ACTA MEDICA SCANDINAVICA

SUPPLEMENTUM 424

ON THE FAT TOLERANCE TEST

BY

GUNNAR ANGERVALL

Accompanies Vol. 176

GÖTEBORG 1964

ACTA MEDICA SCANDINAVICA

SUPPLEMENTUM 424

ON THE FAT TOLERANCE TEST

BY

GUNNAR ANGERVALL

Accompanies Vol. 176

GÖTEBORG 1964

ACTA MEDICA SCANDINAVICA

has been published since 1919 as a continuation of Nordiskt Medicinskt Arkiv founded in 1869 by Axel Key The first volume of Acta Medica Scandinavica is therefore numbered LII (52)

The chief editors have been: Axel Key 1869—1900, C. G Santesson 1901— 1915 I Holmgren 1916—1957 and Birger Strandell 1958 to date

Acta Medica Scandinavica publishes original research papers in the field of internal medicine. Priority will be given to authors from countries which are represented on the editorial board Contributors from those countries should *submit their papers to a representative of the country in question. Contributors* from other countries should send their papers to the Editor at the address below

Submission of a manuscript for publication will be held to imply that the paper has not been published elsewhere and that, if accepted, it will not be republished in any other journal in the same or similar form, without the Editor's writent consent.

Papers will be published in English, French or German at the authors choice, followed by a summary in English.

The manuscripts shall be in final form as submitted. They shall be typewritten with double spacing and broad left hand margin

If a paper exceeds 16 printed pages, the cost for the excess pages shall be borne by the author No paper shall exceed *24 printed pages.*

Subscription

The annual subscription to the journal, covering two volumes each of 6 numbers, is 140 Sw crowns or U.S $ 27.25 *including postage* in the Scandinavian countries and in *Holland 120 Sw crowns.*

Address for subscriptions and all communications

ACTA MEDICA SCANDINAVICA

P O Box 2052 Stockholm 2

Requests for duplicate copies of numbers that have gone astray can only be considered if made within a fortnight of the receipt of the *following number*

ACTA MEDICA SCANDINAVICA
SUPPLEMENTUM 424

FROM MEDICAL DEPARTMENTS I (HEAD: L. WERKÖ, M. D.) AND III (HEAD: T. LINDQVIST, M. D.),
SAHLGRENSKA SJUKHUSET, GÖTEBORG, SWEDEN

ON THE FAT TOLERANCE TEST

BY

GUNNAR ANGERVALL

GÖTEBORG 1964

Translated
by
KLAS MAGNUS LINDSKOG

GÖTEBORG 1944
ELANDERS BOKTRYCKERI AKTIEBOLAG

CONTENTS

Translated
by
KLAS MAGNUS LINDSKOG

GÖTEBORG 1944
ELANDERS BOKTRYCKERI AKTIEBOLAG

I

INTRODUCTION

At Medical Department I Sahl grenska sjukhuset Gothenburg fat tolerance tests — using a standard meal of 200 ml double cream (40 % butter fat content) — have been carried out routinely for the past several years, involving turbidity meas urements among other estimations. The serum turbidity a a rule attained a maximum 4 hours after the fat meal an interval agreeing well with the reported responses to similar fat meals. After 7 hours the turbidity had nearly returned to its original value In 1959 a method for estimation of neutral f t as glyceride-glycerol was published by CARLSON & WADSTRÖM which esti mates neutral fats directly unlike previou methods which estimated neutral fats indirectly as total lipids or total f tty acid less the correspond ng cholesterol and phospholipid com pounds and free fatty acids The former method was immediat ly adopted with the bject of analyzing the serum glyceride-glycerol changes in response t fat meal

Although the majority of workers ha maintained that the turbidity and neutral fat content of serum re associated no attempt has been mad t est blish the nature of this association Thi would provide a means for assessing the value of the serum tu bidity mea urement which by virtue of its simplicity so often is made

As has been the case in a number of previous publications, the present in vestigation included estimation of the cholesterol and phospholipid levels of serum in order to classify the patients in the convential manner and to study the relations of these lipids to glyceride-glycerol in serum at the fat tolerance test

The authors who have studied the reproducibility of fat tolerance tests have merely stated in general terms that in their opinion, the alimentary lipaemia induced by the fat meal was constant in the individual none of them published any numerical data in support of this contention.

In most publi hed investigations involving the use of the fat tolerance test for studying alimentary lipaemia the aim has been to compare and contrast a group of patients with coronary heart disease and a control group Opinions have been expressed about the value of such comparisons. Th fat tolerance of the controls has in most cases proved greater than that of the patients. POMERANZE, BEINFIELD & CHESSIN (1954) and KUO JOYNER, & REINHOLD (1956) have pointed out the fact verified by thi writer that hypercholesterolaemia I r se is a factor that tends to prolong

CHAPTER II

SURVEY OF THE LITFRATURE

As long ago as 1622 ASELLIUS discovered that food absorbed by the small intestine was poured out into the mesenteric lymphatics and made these vessels visible by their milky appearance In 1650 PECQUET succeeded in tracing these vessels to the great veins of th neck, and in 1663 BOYD found that the blood serum itself was made milky after a meal. Rather more than a century later in 1774 HEWSON demonstrated that this milky fluid contained fat and also that under the microscope it showed exceedingly fine particles And in 1887 EDWARDS making his observations in a dark field microscope first described these particles as chylomicrons. (After GAGE & FISH 1924)

NEUMANN (1907) seems to have pioneered the study (by chylomicron counts) of quantitative lipid changes in the blood following a fat load.

Over the years more and more refined methods for determination of fat in blood have been developed The ensuing survey of the literature will be confined mainly to publications relevant to the present investigation namely those dealing with fat loading by the oral rout of non radioactive fat

F t Loading

Th composition of th fat meal ha ried widely and each investigator had to have his own control series Apparently the most common practice has been to administer the fat as a mixed meal implying that the patient is loaded with a roughly estimated amount of fat together with carbohydrates and protein in the form of say a breakfast of bacon and eggs cream, bread and butter etc Whenever fat only has been given, it has generally consisted of cream or olive oil

The effect of carbohydrates upon the lipaemia attending fat meals in dogs was studied by BANG (1918) When bread was substituted for the meat in the meals given the dogs he occasionally observed that the degree of alimentary lipaemia became lower than after fat plus meat. ALBRINK & MAN (1956) gave human subjects glucose 1 hour and $^1/_2$ hour before as well as $1^1/_2$ hours after a fat meal finding that this reduced or even eliminated the serum triglyceride rise (See also MAN & ALBRINK 1956) BERKOWITZ, LIKOFF & SPITZER (1950) reported that a flatter triglyceride curve resulted when glucose was given simultaneously with ^{131}I labelled triolein than when no glucose was given

Little is known about the effect of protein on the lipaemia induced by fat In BANG's experiments on dogs meals of fat plus meat frequently

the alimentary *lipaemia induced by a* fat meal In the majority of published investigations serum turbidity was measured the higher turbidity in atherosclerosis usually being interpreted as higher lipaemia although it could also represent a higher visible fat content i e chylomicron fat In the present investigation therefore particular attention was paid to whether the estimations of chylomicron lipids and *turbidity disclosed* any difference between subjects with and *without* atherosclerosis

There exist no generally accepted definitions of the concepts hypercholesterolaemia and hyperlipaemia In the present investigation a hyperlipaemic will apply to a subject who on at least two occasions, has fasting serum with a glyceride-glycerol level of at least 2 mMol/l as well as a turbidity exceeding 0 10 In other words the writer wishes to retain the concept *of serum lactescence* a lactescent serum being one with a turbidity exceeding *0 10 Normoglyceridaemics* have in turn been classified into normocholesterolaemics and hypercholesterolaemics *the cholesterol level* of the latter s fasting serum on at least two occasions exceeding 300 mg %

The terms atherosclerotics and non atherosclerotics are used in the literature with somewhat varying connotations. Here atherosclerosis will have the following implications

1 Classical angina pectoris *and my*ocardial infarction

2 Angiographic manifestations of atherosclerosis

3 Intermittent claudication with positive oscillometry

The value of the fat tolerance test should be judged by whether it causes say groups with and without clinical atherosclerosis with similar lipid factors of fasting serum to exhibit any differences after a fat meal

Aims of the investigation

To assess the reproducibility especially in hypercholesterolaemics and hyperlipaemics of the alimentary lipaemia induced by a standard fat meal in terms of serum turbidity glyceride-glycerol cholesterol and phospholipids determined both immediately before and 4 hours after the test meal

To study these serum lipid factors before as well as 4 and 7 hours after a standard fat meal in (a) controls (b) hypercholesterolaemics and hyper*lipaemics classified into subgroups* with and without clinical athero*sclerosis and (c) normoglyceridaemic* normocholesterolaemics with clinical *atherosclerosis (males only)*

To study the distribution of serum neutral fats on the chylomicron phase and subnatant at the fat tolerance test and in so doing to analyze the association between neutral fats and turbidity

To compare the same subject s alimentary lipaemias in response to the standard fat meal when he is on a diet containing predominantly saturated fats and when he is on a diet containing predominantly polyunsaturated fats

no change in fat tolerance when the fat content of the diet was raised from 40 to 54 per cent for three weeks. BRONTE-STEWART & BLACKBURN (1958) reported that whereas of 11 patients on a fat restricted (less than 30 %) diet had "high curves 12 other patients on an unrestricted diet included 2 with low curves and 10 with "high curves ALBRINK & NEUWIRTH (1960) stated that the alimentary rise was reduced after to 7 days of starvation. Pointing out that the Bantu the Cape Coloured and the European populations of Cape Town consume 17 45 and 60 per cent respectively of their total calories as fat, BOUCHIER & BRONTE-STEWART (1961) could discern no difference between these groups in their tolerance to a test meal of fat The authors did not specify the caloric intake or the composition of the fat in the diet The significance of fats of the poly unsaturated type was demonstrated by BRONTE-STEWART & BLACKBURN (1958) whose patients with ischaemic heart disease and "high curves after merely a week on maize oil had their fat tolerance altered so that their test curves became indistinguishable from those of the controls It has been observed that the shape of the fat t lerance curves (turbidity) is dependent upon previou dietary fat intake (JONES & DOBRILOVIC 1963).

Work ad fat tol rance NIEMEN (1931) stated that f t loading increased the serum lipid level of normal subjects at rest (in bed) by 4 per cent and the maximum was attained after 4 hours while the corresponding figures were 21 per cent and 3 hours for a group of subjects at work (under graduates and nurses) GAGE & FISH (19 4) recorded higher chylomicron counts after fat loading in persons at work than in persons at rest unlike MARDER *et al* (105) who reported the opposite result. BABBITT (1956) maintained that limited physical activity seemed a relatively unimportant factor the fat tolerance curves of his bed and ambulant patients showed no obvious optical density differences. BILLIMORIA *et al* (1059) found that alimentary lipaemia occurred earlier in exercising than in resting subjects. McDONALD & FULLERTON (1060) found that the increase in plasma turbidity after a fat rich meal was lower in ambulatory patients than in patients confined to bed

COHEN & GOLDBERG (1960) studied the lipaemia induced by fat loading in healthy undergraduates both during rest and during exercise (a 0 4 kilometre walk in about ½ hours) noting that the serum turbidity was significantly lower during exercise NIKKILA & KONTTINEN (1962) had similar results by estimating neutral fat in serum according to VAN HANDEL & ZILVERSMITH (1957)

Some ther factors have been claimed to affect the fat tolerance For example one cigarette per hour caused the chylomicron count to rise in a group of young subjects but not in two elderly subject (MARDER *et al* 193) When habitual smokers were tested with a fat meal the postprandial rise in serum neutral fat was lower in the smoking than in the non-smoking group (KONTTINEN & RAJASALMI 1963)

produced a higher degree of lipaemia than fat alone although *meat alone* did not give lipaemia BING & HECKSCHER (1924) found that when meat was given together with fat the *maximum of alimentary lipaemia* occurred earlier and disappeared faster than when fat was given alone SULLIVAN (1962) found a prolongation and intensification of lipaemia in 4 out of 6 human subjects after protein and fat ingestion as compared with fat ingestion alone

Usually the fat dose has fallen in the range from 0 5 to 4 g per kg body weight or a standard meal as a rule containing 50 to 100 g of fat (as much as 204 g by POMERANZE BEINFIELD & CHESSIN 1954) has been given No *association has been demonstrated* between body weight and the duration of lipaemia after a standard fat meal (BARRITT 1956) Very obese subjects exhibited excessive and prolonged lipaemia following a fat load (POMERANZE BEINFIELD & CHESSIN 1954) MAN & GILDEA (1932) gave up to 4 g of fat per kg body weight a dose nauseating many patients but demonstrated no association between the degrees of lipaemia and nausea MORETON (1950) and KINGSBURY MORGAN & SHERVINGTON (1960) on the other hand maintained that nausea was accompanied by slowing of peristalsis and consequently by altered fat absorption in the digestive tract

Time of administration As a rule meals have been given in the morning after 12 to 14 hours fasting A departure from this rule was made by KINGSBURY MORGAN & SHERVINGTON (1960) who gave a high-carbohydrate *low fat meal* 2 or 3 hours before the fat meal itself the reason given by the authors being that the subjects would then be less nauseated by the fat load

BOHM GERNANDT & HOLMGREN (1941) analyzed diurnal variations in the chylomicron count after fat loading and found that it attained a maximum when the fat load was administered during the night

The diet prior to fat loading has a decisive effect upon the results As long ago as 1924 GAGE & FISH found that fat loading was attended by lower chylomicron counts after 144 hours fasting than after 12 hours fasting BLIX (1925) reported that the elevation after fat loading was accentuated when the subject had been merely a few days on a Petrén diet (200—2 0 g of fat daily) but this alimentary accentuation in due course disappeared and the chylomicron count rise returned to initial size Although BLIX discussed adaptation as a possible explanation of this phenomenon he was more inclined to ascribe it to variations in the rate of fat removal from the blood The fat tolerance was improved by 6 weeks isocaloric fat restriction to less than 20 g daily (POMERANZE BEINFIELD & CHESSIN 1954) *Under metabolic ward conditions* HAVEL (1957) obtained appreciable fat tolerance differences in healthy subjects on an isocaloric diet when their daily fat intake per kg body weight was varied from 0 1 to 2 0 g with the highest fat tolerance after the lowest intake

Conversely HORLICK (1957) found

no change in fat tolerance when the fat content of the diet was raised from 40 to 54 per cent for three weeks. BRONTE-STEWART & BLACKBURN (1958) reported that whereas of 11 patients on a fat restricted (less than 20 %) diet had high curves 12 other patients on an unrestricted diet included with low curves and 10 with "high curves". ALDRINK & NEUWIRTH (1960) stated that the alimentary rise was reduced after to 7 days of starvation. Pointing out that the Bantu the Cape Coloured and the European populations of Cape Town consume 1 43 and 60 per cent respectively of their total calories as fat BOUCHIER & BRONTE-STEWART (1961) could discern no difference between these groups in their tolerance to a test meal of fat. The authors did not specify the caloric intake or the composition of the fat in the diet. The significance of fats of the poly unsaturated type was demonstrated by BRONTE-STEWART & BLACKBURN (1958) whose patients with ischaemic heart disease and high curves after merely a week on maize oil had their fat tolerance altered so that their test curves became indistinguishable from those of the control. It has been observed that the shape of the fat tolerance curves (turbidity) is dependent upon previous dietary fat intake (JONES & DOBRILOVIC 1962).

Work and fat tolerance NIKKEN (1931) stated that fat loading increased the serum lipid level of normal subjects at rest (in bed) by 42 per cent and the maximum was attained after 4 hours while the corresponding figures were 34 per cent and 3 hours for a group of subjects at work (undergraduates and nurses). GAGE & FISH (1924) recorded higher chylomicron counts after fat loading in persons at work than in persons at rest, unlike MARDER *et al* (195) who reported the opposite result. BARRITT (1956) maintained that limited physical activity seemed a relatively unimportant factor—the fat tolerance curves of his bed and ambulant patients showed no obvious optical density differences. BILLIMORIA *et al* (1959) found that alimentary lipaemia occurred earlier in exercising than in resting subjects. McDONALD & FULLERTON (1960) found that the increase in plasma turbidity after a fat rich meal was lower in ambulatory patients than in patients confined to bed.

COHEN & GOLDBERG (1960) studied *the lipaemia induced by fat loading in* healthy undergraduates both during rest and during exercise (a 9 4 kilometre walk in about 2 ½ hours) noting that the serum turbidity was significantly lower during exercise. NIKKILA & KONTTINEN (1962) had a milar result by estimating neutral fat in serum according to VAN HANDEL & ZILVERSMITH (1957).

Some other factors have been claimed to affect the fat tolerance. For example one cigarette per hour caused the chylomicron count to rise in a group of young subjects but not in two elderly subjects (MARDER *et al* 1952). When habitual smokers were tested with a fat meal the postprandial rise in serum neutral fat was lower in the smoking than in the non-smoking group (KONTTINEN & RAJASALMI 1963).

"Stress" was responsible for a peak in the chylomicron curve (GAGE & FISH 1924)

Reproducibility of fat tolerance Some workers have studied variations in fat tolerance NISSEN (1931) for example expressed the opinion that a fat load of 1 g per kg body weight consistently gave rise to the same fat tolerance curve in the same normal subject MORETON (1950) and OSMONZINN & WHARTON (1957) concurring made similar observations even when the interval between consecutive fat tolerance tests was up to 6 months Slight variations however have been reported by other workers (MESSINGER & POROSOWSKA 1953 BARRITT 1956) Performing repeated fat tolerance tests in 15 patients with ischaemic heart disease and in 10 controls BRONTE STEWART & BLACKBURN (1958) found considerable variability in response to the same fat load although those who exhibited a high curve continued to do so and vice versa The fat load had the form of a breakfast of bacon and eggs etc and its fat content was less well defined than the fat meals given by the other authors mentioned here DENBOROUGH (1963) found that duplicate fat tolerance tests (70 g fat as a mixed meal) were consistent (triglycerides according to VAN HANDEL & ZILVERSMITH) JONES & DOBRILOVIC (1963) performed 62 fat tolerance tests (breakfast with 30 g fat) in 7 patients and found that the total fatty acid content of the chylomicron fraction did vary with the optical density and that the shape of the fat tolerance curve was dependent upon the previous dietary fat intake as well as on the quality of the test fat

Serum Lipid Changes After Fat Loading

Chylomicron counting seems to represent the very first attempt at quantitative blood fat estimation (NEUMANN 1907) and the method remained in use well into the 1950 s (e g MARDER *et al* 1952 GRÖNER & HILDEN 1955)

Different fats give rise to specific types of chylomicrons (SWANK & LEVY 1952) Triolein gave large distinctive spherical chylomicrons while those seen after tristearin and cream vary in shape and are comparatively small The chylomicrons are small (about 0 5 μ) at the beginning and end of the fat tolerance curve but larger (about 1 μ) at maximal lipaemia (GAGE & FISH 1924) Even though most of the blood fat increase occurs in the form of chylomicrons (GRÖNER & HILDEN 1953) the chylomicron count would seem to be a poor estimate of the serum lipid increase after a fat meal

Turbidity Another way of estimating visible lipids in serum is to measure its turbidity either nephelometrically or spectrophotometrically The former technique directly measures the turbidity as reflected light requiring special instruments and standard solutions — $BaSO_4$ suspension in glycerol — it is the more complex of the two (MORETON 1950 MARDER *et al* 1952 MESSINGER & POROSOWSKA 1953) The latter technique is indirect and measures the degree of light absorption by optically active particles

Serum turbidity has in most cases been measured spectrophotometrically at wavelength 650 mμ with variations in the range 610—700 mμ.

Particles with diameters more than one-quarter the wavelength of visible light (or 0.1 μ) deflect and scatter incident rays of light and give the serum the appearance of turbidity or lipaemia (AHRENS & KUNKEL, 1949). Neutral fat is the only lipid consistently associated with serum lactescence, and the soluble neutral fat of lactescent serum was found to be between the limits 10 and 30.4 mEq/l neutral fatty acids when total fats varied from 16.4 to 478 mEq/l (ALBRINK, MAN & PETERS, 1954).

The turbidity and neutral fat content of serum were as a rule well correlated in BARRITT's (1956) patients. ELORRIETA & SCHETTLER (1958) found closely parallel turbidity and neutral fat values along the ascending portion of the fat tolerance curve after linseed oil but the two diverged along the descending portion; this deviation was less marked after coconut oil or butter. DEWHURST & WHARTON (1957) remarked that serum turbidity was correlated with rise in total lipids in their studies on the tolerance to 1 g of fat per kg body weight. JONES & DOBRILOVIC (1962) is of the opinion that optical density provides a fairly reliable indication of the postprandial changes in chylomicron fatty acid concentration.

Cholesterol. The majority of investigations have shown that the serum cholesterol level remained constant or was negligibly changed after a fat load (BLOOR, 1916; BLIX, 1925; HIRSCH & CARBONARO, 1950; POMERANZE, BEINFIELD & CHESSIN, 1954; ALBRINK & MAN, 1956). Not so HILLER et al (1924), who recorded an average increase of 5 per cent, and BRUGER (1940) with a mean cholesterol rise of 20 mg %, after a fat meal. HAVEL (1957), obtaining no rise in men, claimed that 7 of 8 women showed an increase of at least 15 mg % in very low density lipoproteins. BLOTNER (1935) stated there was no rise in thin persons but a noticeable rise in fat persons. On the other hand NIKKILÄ & KONTTINEN (1962) found a significant decrease 6 hours after loading with 55 g butter fat in healthy soldiers.

KINGSBURY & MORGAN (1960) reported different cholesterol responses after 15 different oils; while some of them induced a serum cholesterol (and phospholipid) rise of approximately 20 mg%, others instead induced a reduction.

Phospholipids. There seems to be substantial agreement that fat loading raises the amount of serum phospholipids. MAN & GILDEA (1932) noted a slight phospholipid increase 3 hours after 60 g of fat but an 18 per cent rise after between 3.4 and 4 g fat per kg body weight. POMERANZE, BEINFIELD & CHESSIN (1954) recorded phospholipid increases between 29 and 94 mg% five hours after a fat meal, the rise being lowest in the groups: extremely obese male individuals (20—40 years) with 29 mg% and normal males with normal lipids (20—40 years) with 33 mg% and highest — 94 mg% — among males over 70 years with

clinical evidence of atherosclerosis" the group showing the highest alimentary lipaemia ALBRINK & MAN (1956) on the other hand obtained no phospholipid increase after a load of 60 g fat in a mixed meal Recently other authors have also observed increase of phospholipids in similar trials (TALBOTT & KEATING 1962 SKIBBE & HAUDE 1963)

HAVEL (1957) recorded increases of both high density and low density lipoprotein phospholipids. A delayed fall was due to the persistence of the increased concentration of high density lipoprotein phospholipids after the visible lipaemia had disappeared

Total fats neutral fat Several previously quoted authors have estimated total fats at fat tolerance tests (HERZSTEIN *et al* 1953 BARRITT 1956 OSMON ZINN & WHARTON 1957) Others have estimated total fatty acids (POMERANZE, BEINFIELD & CHESSIN 1954 HORLICK 1956 1957) Neutral fat has been calculated as total fat or total fatty acids less the corresponding cholesterol and phospholipid compounds and free fatty acids (*MAN & ALBRINK 1956 BARRITT 1956 EGGSTEIN* & SCHETTLER, 1958 TALBOTT & KEATING 1962 SKIBBE & HAUGE 1963)

Direct estimation of neutral fat has been enabled by chromatographic separation This technique was introduced for clinical use by CARLSON & WADSTRÖM (1956) A similar method for neutral fat was worked out by VAN HANDEL & ZILVERSMITH (1957) With this method *NIKKILÄ & KONTTINEN* determined neutral fat in serum in 20 healthy soldiers after a breakfast with 55 g fat Fasting neutral fat in serum *was 98 mg% and increased 4 hours* after the fat load to 175 mg% DENBOROUGH (1963) gave 70 g fat as a mixed meal to controls Neutral fat in fasting serum was 110 mg% and increased to about 240 mg% 3½ hours after the fat meal The author found a *correlation between the fasting and* 3½ hours values as well as between the fasting and the 7½ hours values Also between the maximal and fasting triglyceride values there was a significant correlation

Age and fat tolerance Investigations into the *association between age* and fat tolerance have had divergent results. BECKER, MEYER & NECHELES (1949 1950) stated that the chylomicron count after a fat meal rose more in a group of 30 subjects over 50 (mean age 70 years) than in a group of 30 subjects under 50 (mean age 18 years) MARDER *et al* (1952) reported similar results of turbidity measurements Despite using the same chylomicron counting principles (FRAZER & STEWART *1937) as BECKER, MEYER & NECHELES, GRÜNER & HILDES* (1953) were unable to verify their findings but found instead that older subjects (over 50 years) had a significantly lower chylomicron count in response *to fat loading* than younger subjects Notably however while the test meal consisted of 0 5 g margarine per kg body weight plus tea and bread in the two former investigations it was a *standard meal* of 150 ml cream with a butter fat *content* of 13 *per* cent in the latter study In both the control group and

the coronary disease group studied by SCHWARTZ, WOLDOW & DUNSMORE (195) the turbidity of serum after a fat load was higher in subjects over 40 years of age than in subjects under 40

HERZSTEIN WANG & ADLERSBERG (1953) determined total fats after fat loading with 1 g fat (as 40 per cent cream) per kg body weight obtaining similar increases after hours in young (mean age 24 ± years) and old (mean age 6 years) subjects without metabolic disease or evidence of atherosclerosis. After 6 hours the total fats had returned to their initial level in the young group but remained enhanced in the old group POMERANZE, BEINFIELD & CHESSIN (1954) found an elevated and prolonged fat tolerance curve (total fatty acids) in 6 males over 70 years of age with evidence of atherosclerosis, as compared with corresponding curves for other males between 20 and 40 years of age The turbidity measurements and total fat estimations in BARRITT's (1956) investigation disclosed no significant correlation between age and the degree of lipaemia induced by a fat meal. But he remarked that young men showed a tendency to greater lipaemia. Nor did BOUCHIER & BRONTE-STEWART (1961) discover any turbidity difference between subjects over and under 40 years of age in conjunction with fat tolerance tests. BROWN HEALIN & DOYLE (1961) found that after a fat meal healthy middle-aged men 38—63 years of age cleared fat in serum more slowly than healthy male students

Body weight and fat tolerance BARRITT (1956) observed no significant correlation between body weight and the duration of lipaemia in response to a standard fat meal. Similarly BRONTE STEWART & BLACKBURN (1958) were unable to find any association between skinfold thickness and fat tolerance Conversely TANNHAUSER (1947) stated that postprandial lipaemia might persist for unusually long periods in obese persons. The latter finding is in agreement with those of POMERANZE, BEINFIELD & CHESSIN (1954) who tested the fat tolerance of extremely obese male individuals (20—40 years) and found that their lipaemia (total fatty acids) was considerably prolonged but not elevated compared with that in normal males of corresponding age The fat tolerance rose appreciably after weight reduction had been enforced.

Atherosclerosis and fat tolerance. MORETON (1947 1948 1950) postulated that a relationship existed between chylomicrons and atherosclerosis So did BECKER, MEYER & NECHELES (1950) against the background of the longer and higher chylomicron curve they observed in old (over 50 years) than in young (under 50 years) subjects. SCHWARTZ, WOLDOW & DUNSMORE (1952) reported that the turbidity of serum was greater in patients given a fat meal 1 to 8 weeks after onset of myocardial infarction than it was in the control group (convalescents after acute infections and minor surgical interventions) both before as well as 3 and 5 hours after a meal of cream and carbohydrates.

WOLDOW CHAPMAN & EVANS (1954)

tested the fat tolerance (standard cream meal) of 21 patients with evidence of coronary disease and noted that the lipaemia in this group was increased in both degree and duration compared with that in a control group However while the former group had a mean age of 55 years the mean age of the latter was only 31 years

POMERANZE BEINFIELD & CHESSIN (1954) reported that hypercholesterolaemics without other evidence of disease (20—40 years) and older males (over 70 years) with atherosclerosis showed a response to fat loading in the form of higher and longer lipaemia (total fatty acids) by comparison with young men (20—40 years) with normal blood lipids BARRITT (1956) reported significantly higher fasting values for both turbidity and total lipids in men with coronary artery disease than in controls The greatest difference was observed 5 and 7 hours after a load of 60 g fat After a meal of 0 6 g heavy cream per lb body weight KUO JOYNER & REINHOLD (1956) recorded higher values at the 5th than at the 3rd hour *in 6 of 7 hyperlipaemics 10 of 12 hypercholesterolaemics 10 of 15* atherosclerotic subjects (coronary heart disease) and 2 of 10 controls BRONTE STEWART & BLACKBURN (1958) $5\frac{1}{2}$ and 7 hours after a 100 g fat breakfast found significantly higher serum turbidity in patients with ischaemic heart disease than in controls (schizophrenics) but a marked overlap between the groups existed MITCHELL & BRONTE STEWART (1959) made similar observations when they compared turbidities in patients with ischaemic heart disease and in age-matched controls (convalescents) after a 75 g fat breakfast. Similar results were reported by BOUCHIER & BRONTE-STEWART (1961) HORLICK (1956 1957) found that the total fatty acid level in cases of coronary atherosclerosis admittedly remained elevated *longer than in the* controls (young healthy males) but that a high fat test meal failed to uncover or exaggerate lipid metabolic defects in individuals with atherosclerosis

Summing up it may be stated that when hypercholesterolaemics and subjects with clinical atherosclerosis are subjected to fat tolerance tests their *fat tolerance curve will generally be higher and longer than in controls*

CHAPTER III

CLINICAL MATERIALS

Over the years 1959 through 1962 the subjects for the present investigation were selected at Medial Departments I and III and the Orthopaedic Department, Sahlgrenska Sjukhuset, Gothenburg In addition the controls included some healthy subjects.

Classification

The groups of subjects may be classified as follows (see Table 1)

A Groups in which the individual was studied by repeating the fat tolerance test once or twice

1 The subject was on the same diet when two fat tolerance tests were performed, these observations were also used for assessing the reproducibility (Cf. Table 10 p 30)

The cholesterol phospholipid and glyceride-glycerol levels and turbidity were determined in serum specimens taken immediately before and 4 hours after a standard fat meal.

It was ensured that the patients were on the same diet preceding the duplicate fat tolerance tests and were free from infections or other diseases. The duplicate fat tolerance tests were performed on 48 in-patients and 9 out patients, the interval between tests being 6 3±0 3 days in 51 cases and from 21 to 142 days in the remaining 6 cases. The age of the subjects ranged from 25 to 68 years and averaged 47 years. 31 were up to and 26 over 50 years of age. Both these age groups included similar proportions of hyperlipaemics and hypercholesterolaemics

2. The subject was put on a different diet at least 6 weeks prior to the repeated fat tolerance test and the following comparisons were made.

The response to the fat meal of subjects on a diet with mainly saturated fats was compared with that of the same subjects on a diet with mainly poly-unsaturated fats. The intention was not to disclose any reductions in serum lipids or in the turbidity but rather to compare the courses of the responses to the standard fat meal on these diets In 32 cases this comparison could be made between sera before and 4 hours after the fat meal and in 15 cases before as well as 4 and 7 hours after the fat meal.

The change from the subject s ordinary diet with saturated fats to one with poly unsaturated fats was usually made in two stages. The first stage was restriction of saturated fats. Comparisons could in 13 cases be made between the response on ordinary diet and that on a diet with restriction of saturated fats

In only 6 cases the fat test meal was given on ordinary diet on diet with restriction of saturated fats and on the

tested the fat tolerance (standard cream meal) of 21 patients with evidence of coronary disease and noted that the lipaemia in this group was increased in both degree and duration compared with that in a control group However while the former group had a mean age of 55 years, the mean age of the latter was only 31 years

POMERANZE BEINFIELD & CHESSIN (1954) reported that hypercholesterolaemics without other evidence of disease (20—40 years) and older males (over 70 years) with atherosclerosis showed a response to fat loading in the form of higher and longer lipaemia (total fatty acids) by comparison with young men (20—40 years) with normal blood lipids. BARRITT (1956) reported significantly higher fasting values for both turbidity and total lipids in men with coronary artery disease than in controls The greatest difference was observed 5 and 7 hours after a load of 60 g fat After a meal of 0 6 g heavy cream per lb body weight KUO JOYNER & REINHOLD (1956) recorded higher values at the 5th than at the 3rd hour in 6 of 7 hyperlipaemics, 10 of 12 hypercholesterolaemics 10 of 15 atherosclerotic subjects (coronary heart disease) and 2 of 10 controls. BRONTE STEWART & BLACKBURN (1958) 5½ and 7 hours after a 100 g fat breakfast found significantly higher serum turbidity in patients with ischaemic heart disease than in controls (schizophrenics) but a marked overlap between the groups existed. MITCHELL & BRONTE STEWART (1959) made similar observations when they compared turbidities in patients with ischaemic heart disease and in age-matched controls (convalescents) after a 75 g fat breakfast Similar results were reported by BOUCHIER & BRONTE-STEWART (1961) HORLICK (1956 1957) found that the total fatty acid level in cases of coronary atherosclerosis admittedly remained elevated longer than in the controls (young healthy males) but that a high fat test meal failed to uncover or exaggerate lipid metabolic defects in individuals with atherosclerosis

Summing up it may be stated that when hypercholesterolaemics and subjects with clinical atherosclerosis are subjected to fat tolerance tests their fat tolerance curve will generally be higher and longer than in controls

TABLE [illegible] Fat tolerance tests [illegible]

Sex	Group	n	Age years	Cholesterol in mg per cent S_0	Cholesterol $S_4 - S_0$	Phospholipids in mg per cent S_0	Phospholipids $S_4 - S_0$	Triglycerol in mMol/l S_0	Triglycerol S_4	Turbidity at 650 mμ S_0	Turbidity S_4
Male	Controls	42	50.9	233	+1.1	280	+20	1.14	2.01	0.10	0.33
			2.0	*8*	*1*	*6*	*2*	*0.05*	*0.2*	*0.01*	*0.04*
	Hypercholesterolemia without clinical atherosclerosis	11	45.9	358	0.7	311	+17	1.50	2.54	0.12	1.09
			2.3	*5*	*6.6*	*18*	*8*	*1.11*	*0.32*	*0.05*	*0.19*
	Hypercholesterolemia with clinical atherosclerosis	28	48.3	323	+2.0	[illegible]	+24	1.09	3.30	0.10	0.80
			2.8	*13*	*3.3*	*18*	*6*	*0.09*	*0.28*	*0.05*	*0.11*
	Hyperlipaemia without clinical atherosclerosis	14	44.5	253	+6.6	357	+39	4.14	7.22	0.30	3.11
			2.7	*33*	*3.1*	*31*	*7*	*0.73*	*0.70*	*0.08*	*0.18*
	Hyperlipaemia with clinical atherosclerosis	8	53.1	259	−3.4	313	+18	3.85	6.27	0.17	1.40
			3.6	*22*	*1.8*	*31*	*9*	*0.76*	*1.31*	*0.01*	*0.32*
	Normocholesterolemic normoglyceridemic atherosclerotics	15	53.4	264	+2.4	283	+.5	1.53	3.14	0.08	1.08
			3.3	*4*	*4.9*	*8*	*7*	*0.13*	*0.27*	*0.01*	*0.31*
Female	Controls	26	42.8	270	+1.4	375	+20	1.00	1.57	0.08	0.45
			2.4	*6*	*1.4*	*7*	*4*	*0.06*	*0.14*	*0.01*	*0.01*
	Hypercholesterolemia without clinical atherosclerosis	26	48.7	383	+0.9	319	+26	1.41	2.35	0.08	0.65
			2.3	*56*	*4.1*	*18*	*11*	*0.14*	*0.34*	*0.05*	*0.09*
	Hypercholesterolemia with clinical atherosclerosis	18	54.9	340	+7.4	321	+26	1.74	2.78	0.09	0.54
			2.1	*14*	*2.1*	*18*	*4*	*0.10*	*0.21*	*0.01*	*0.03*
	Hyperlipaemia without clinical atherosclerosis	6	40.8	357	+2.0	330	+27	2.94	4.09	0.18	1.03
			3.9	*32*	*1.6*	*49*	*5*	*0.43*	*0.77*	*0.03*	*0.30*
	Hyperlipaemia with clinical atherosclerosis	8	54.0	420	+3.4	406	+38	4.94	7.39	0.33	2.03
			2.3	*12*	*6.3*	*13*	*9*	*1.96*	*2.34*	*0.11*	*0.31*

S_0 and S_4 = serum before and 4 hours after the fat load.
Figures in italics are standard error of the mean.

TABLE 1 *Survey of cli cal Series*

Fat tolerance tests group		Controls	Hypercholesterolaemia 0 ath.	Hypercholesterolaemia +ath.	Hyperlipaemia 0 th.	Hyperlipaemia + th.	Normochol normoglyc +ath		Total
A *Individual studies*									
1 duplicate	♂	3	9		12		3	29	57
	♀	1	21		5		1	28	
2. repeated on different diets									
a. N and PU $S-S$	○	—		3	6	0	—	17	3
	♀	—		4	1	3	—	15	
N and PU $S-S-S$	♂	—					—	8	15
	♀	—	3	1	0	3	—		
b N and FR $S-S$	♂	1	1	0	2	3	1		13
	♀	—		1	0	3	—	6	
c N FR and PU	♂	—	1	0	0	2	—	3	6
	♀	—	3	0	0	)	—	3	
B Group studies									
1 $S-S$-series	♂	4.	11	28	14	8	13	116	10
	♀	36	56	18	6	8	—	94	
.. $S-S-S_7$-series	♂	4	7	18	6	4	8	68	115
	♀	20	11	8	4	4	—	47	
3 S_0, S and substants	♂	36	8	18	9	0	8	88	166
	♀	34	22	10	8	0	—	78	
4 S_7 and substant	♂	12	3	9	2	2	3	31	52
	♀	9	3	3	4	2	—	21	

Ath = clinical atherosclerosis. N = ordinary diet. FR = diet restricted in saturated fat. PU = FR-diet and poly unsaturated f t added. S_0, S_4, S_7 = serum before and 4 and 7 hours after the fat meal.

latter diet with poly unsaturated fats added

B Groups studied as such in which each subject is represented by a single fat tolerance test These groups were subdivided into male and female con trols, hypercholesterolaemics hyperli paemics and subjects with and with out clinical manifestations of athero sclerosis A *discrete group comprised* normoglyceridaemic and *normocholes*terolaemic males with clinical atheros clerosis The various groups in this category were[1]

1 The S_0-S_4 Series (210 subjects) in *which one serum specimen taken im*mediately before (S_0) and another 4 hours after the fat meal (S_4) were *analyzed (see Table 2)*

[1]) *Clinical data nd the result of the f t toler ance test for each patient will be supplied on request from any terested reader*

TABLE 3 [illegible]

Sex	Group	n	Age years	Cholesterol in mg per cent K_0	Cholesterol ± %	Phospholipids in mg per cent S	Phospholipids ± %	Triglyceride glycerol in mMol/l S_0	Triglyceride glycerol S	Turbidity at 650 mμ S_0	Turbidity S
Male	Controls	45	50.9	237	3.1	250	+20	1.14	2.01	0.10	0.33
			2.8	*6*	*1.7*	*6*	*3*	*0.08*	*0.12*	*0.01*	*0.04*
	Hypercholesterolaemia without clinical atherosclerosis	11	43.9	256	0.7	215	17	1.60	2.64	0.12	1.03
			3.5	*11*	*6.8*	*16*	*3*	*1.11*	*0.33*	*0.03*	*0.19*
	Hypercholesterolaemia with clinical atherosclerosis	29	49.5	332	+2.0	288	+24	1.89	3.30	0.10	0.88
			3.5	*13*	*3.3*	*18*	*4*	*0.29*	*0.33*	*0.03*	*0.11*
	Hyperlipaemia without clinical atherosclerosis	14	44.5	303	+8.6	327	+29	4.84	7.33	0.30	2.11
			2.7	*24*	*6.1*	*21*	*7*	*0.72*	*0.78*	*0.08*	*0.18*
	Hyperlipaemia with clinical atherosclerosis	8	53.1	288	−2.4	313	+18	3.85	6.37	0.17	1.40
			3.6	*25*	*4.8*	*31*	*9*	*0.70*	*1.31*	*0.01*	*0.33*
	Normocholesterolaemic normoglyceridaemic atherosclerosis	13	53.4	264	+2.4	262	+23	1.63	3.14	0.06	1.08
			3.8	*4*	*1.9*	*6*	*7*	*0.13*	*0.37*	*0.01*	*0.24*
Female	Controls	36	43.6	279	+1.4	274	+20	1.00	1.67	0.04	0.42
			2.4	*8*	*1.5*	*7*	*3*	*0.08*	*0.14*	*0.01*	*0.04*
	Hypercholesterolaemia without clinical atherosclerosis	46	48.7	363	+9.8	349	+28	1.41	2.33	0.06	0.63
			2.3	*16*	*4.1*	*14*	*11*	*0.14*	*0.31*	*0.03*	*0.09*
	Hypercholesterolaemia with clinical atherosclerosis	18	53.9	349	+7.4	331	+20	1.74	2.78	0.09	0.54
			2.1	*18*	*2.1*	*10*	*2*	*0.10*	*0.21*	*0.01*	*0.03*
	Hyperlipaemia without clinical atherosclerosis	8	48.8	287	+2.8	330	+27	2.94	4.09	0.15	1.06
			3.8	*13*	*4.6*	*26*	*3*	*0.42*	*0.77*	*0.03*	*0.30*
	Hyperlipaemia with clinical atherosclerosis	8	38.0	470	+8.4	406	+28	4.84	7.29	0.22	2.03
			2.3	*12*	*8.3*	*18*	*9*	*1.08*	*2.34*	*0.11*	*0.34*

* S_0 and S = serum before and 4 hours after the fat load.
Figures in italics are standard error of the mean

Controls This group comprised both healthy subjects and patients with orthopaedic disorders whose history and clinical picture exhibited no clinical manifestation of atherosclerosis gallstone disease hypertension[1]) or *other non-orthopaedic disease* The clinical study included a general physical examination Ecg haemoglobin concentration, E.S R and estimation of urinary sugar and albumin Moreover oscillometry was done in every case and especial attention was *paid to the* presence of arcus corneae xanthelasma and xanthoma None of the controls had received any dietary supplement of poly unsaturated fats or otherwise altered his regular diet

The majority of the controls were orthopaedic patients Indeed 31 of the *42 male controls suffered from ortho*paedic conditions 10 of them from back pain of various types predominantly sciatica The corresponding figures for the female controls were 20 of 36 and 13 of 26 None of the controls had severe pains — practically all *could walk to the lipid laboratory*

The E S R was between 10 and 20 mm in 11 and over 20 mm in 3 of the 78 controls. Among 3 men and one woman between 80 and 90 years *of age all of whom felt fit* took daily walks etc one had oscillometric ab*normalities* and a PR interval of 0 80 seconds, while the Ecg revealed that another had auricular fibrillation the third bundle-branch block and the fourth diphasic T waves

The controls were selected with a view to having all age decades between 20 and 90 years represented (See Table 3) Nevertheless it turned out *that the proportion of females up to* 50 years of age was significantly higher than that of men ($\chi^2 > 3$ 84) No difference in lipids or turbidity could be demonstrated between these age groups for either males or females Fig 1 shows the glyceride-glycerol levels and *turbidities of fasting serum the re*spective means for men and women being 1 00 and 1 14 mMol/l and 0 10 and 0 075 turbidity units (Cf Tables *2* and 10)

The mean cholesterol and phospholipid levels of fasting serum were somewhat high for both male (?55 and -70 mg%) and female (250 and 2 5 mg %) controls When these groups were classified according to whether or not consanguineous relatives had gallstone disease coronary *affection or diabetes*

[1]) except 3 cases. 36 years male 160 105 63 years male 20 100 d 72 years female 160 105

TABLE 3. *Age distribution / control*

	?0 – 30	31 – 40	41 – 50	51 – 60	61 0	70 years	T t l
Men	7	6	7	10	0	0	4
W men	6	8	12	7	1		36
T tal	13	14	19	17		8	8

mellitus (i e a positive or negative family history) the mean lipid levels and turbidities set out in Table 4 were obtained. Evidently these values did not differ significantly for the groups with positive and negative family histories The cholesterol level of fasting serum exceeded 300 mg in 12 of the female controls, 10 of whom were ≥ 45 years of age, i.e during and after the menopause

The association, if any between sciatica and serum lipids was studied by comparing 18 subjects (9 men and 9 women) classified as healthy controls and 20 patients (11 men and 9 women) with a diagnosis of sciatica, all being below 60 years of age While the mean serum cholesterol levels for these two groups — 256±9 mg and 277±13 mg respectively — were not significantly unequal the corresponding glyceride-glycerol levels — 0 80±0 08 mMol/l) and 1 22±0 11 mMol/l — did differ significantly

) 95% range calculated on log glyceride-glycerol 0 3–1 mMol l.

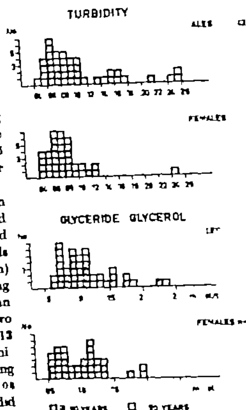


Fig 1 Frequency distribution of turbidity and glyceride-glycerol in serum of fasting controls

TABLE 4 *Lipids and turbidity in fasting serum from controls classified according to family history of gallstone disease coronary disease or diabetes mellitus*

	History		Cholesterol mg%	Phospholipids mg%	Glyceride-glycerol mMol/l	Turbidity
Men	negative	32	248± 7	240± 7	1 14±0 07	0.10±0 01
	positive	16	276±11	253± 8	1.13±0.12	0.10±0 02
Women	negative	23	270±12	273±10	1.03±0 07	0.09±0 04
	positive	13	281±12	279±10	0 90±0.10	0 09±0 01

No significant lipid differences could be demonstrated in fasting serum between male and female controls with and without positive family history

TABLE 5 *Familial and clinical histories of hypercholesterolaemics, hyperlipaemics and normocholesterolaemic normoglyceridaemic atherosclerotics*

History		Hypercholesterolaemics				Hyperlipaemics				Normo-chol.[1]
		0 ath		+ ath		0 ath		+ ath		+ ath
	n =	11 ♂	26 ♀	*8 ♂	18 ♀	14 ♂	6 ♀	8 ♂	8 ♀	13 ♂
Heredity										
Hyperlipaemia and/or hypercholesterolaemia		4/4 *)	15/15	2/	4/5	2/2	/3	0/1	3/3	0/0
Myocardial infarction		6	15	10	11	1		4	3	4
Diabetes mellitus		1	8	0	1	2	1	1		3
Clinical picture										
Xanthoma		5	10	0	3	2	0	1		1?
Xanthelasm		1	8	4	4	0	0	0	4	
Arcus corneae		4	11	10	5	3	1	3		3
Gallstone disease		0	6	4	5	1	1	1	3	1
Hypertension		3	5	14	11	5	3	3	4	5
Ecg during and after exercise		7	13	8	5	7		4	0	3
Angiography		1	1	10	9	3		0		0

[1]) Normocholesterolaemic normoglyceridaemics.

*) no. positive/no. examined

Clinical groups. Hypercholesterolaemics, hyperlipaemics and atherosclerotics not belonging to either of these groups were subjected to fat tolerance tests. Patients with an active disease not associated with atherosclerosis were not included. Thus patients with active gallstone disease, gastroduodenal ulceration and other gastrointestinal disorders were eliminated. So were patients with a known infection such as the "common cold", pneumonia or rheumatic disorders and convalescent surgical patients. Great care was taken not to include patients whose diet had been changed, e.g. for voluntary weight reduction by fat restriction and/or addition of poly unsaturated fats.

Frequencies of clinical data of significance for blood lipid changes are given in Table 5 which provides information on heredity, blood pressure, gallstone disease, occurrence of arcus corneae, xanthoma and xanthelasma.

Hypercholesterolaemics. The principal for selection of hypercholesterolaemics without and with atherosclerosis differed. The former group was selected because xanthelasma, xanthoma or arcus corneae had called for analysis of serum lipids. Moreover, the family history was penetrated in cases where some lipid abnormality had been demonstrated and if possible the relatives were examined. The group of hyper

cholesterolaemics with atherosclerosis was selected because the atherosclerotic disease had necessitated serum lipid analysis. Clinical particulars for the two groups are presented in Table 5. The table reveals that the incidence of hypertension was significantly higher in the atherosclerotic than in the non-atherosclerotic group a difference associated with the inclusion of many patients with stenosis of the renal artery. The frequencies of other clinical factors exhibited no major differences. Abnormal glucose tolerances were equally common in the two groups.

The cholesterol and phospholipid levels of fasting serum tended to be higher in the non-atherosclerotic groups than in the atherosclerotic ones. This difference was significant when men and women were consolidated into a single group and was most likely due to the principles of selection. The converse situation applied to glyceride-glycerol that is the combined groups of atherosclerotics showed the higher serum glyceride-glycerol level.

The atherosclerotic and non-atherosclerotic groups of hypercholesterolaemics differed in yet another way namely with respect to the higher mean age of the former (significantly for men plus women and for women alone). Accordingly age (±1 year) and sex matched groups were studied. These two groups (8 men and 11 women in each) exhibited no differences in the cholesterol, phospholipid and glyceride-glycerol levels of fasting serum (or serum 4 hours after a fat meal).

Hyperlipaemics. For the past several years estimations of cholesterol in fasting serum have systematically been combined with serum turbidity measurements even mild turbidity increases (>0 14) leading to estimation of the glycerides. The selection of hyperlipaemics with and without clinical atherosclerosis was based on criteria similar to those applying to the corresponding groups of hypercholesterolaemics. The frequencies of the clinical factors presented in Table 5 were similar in the hyperlipaemics with and without clinical atherosclerosis.

The four women with xanthelasma — reportedly a rare phenomenon in hyperlipaemia — all had serum glyceride-glycerol levels between and 3 mMol/l. The hyperlipaemic groups showed a significantly lower frequency of xanthelasma than the hypercholesterolaemic groups. These categories did not deviate in regard to the other clinical factors included in Table 5.

Normoglyceridaemic normocholesterolaemics with atherosclerosis. To this group were assigned those patients who could not be included among either the hypercholesterolaemics or the hyperlipaemics, although in most cases numerous, often serial analyses of lipids in fasting serum had been carried out. This group exhibited no characteristic deviations from the hypercholesterolaemic and hyperlipaemic groups with respect to the clinical factors included in Table 5. The mean glyceride-glycerol level of fasting serum in this group significantly exceeded that for the controls (1 33 and 1 14 mMol/l respectively).

2. *The S – S – S Series* (115 sub-

TABLE 6. *Fat tolerance t sts the S – S₄ – S₇-Series*

Sex	Group	n	Age Years	Cholesterol mg per cent			Phospholipids mg per cent			Glyceride-glycerol mMol/1			Turbidity		
				S	S_4	S	S	S	S	S	S	S_7	S	S	S_7
Male	Controls	4	46.3	_36	250	_31	_30	*83	73	1 18	4	1 37	0 08	0 67	0.31
			3 1	*3*	*3*	*?*	*3*	*9*	*3*	*0 10*	*0 19*	*0 14*	*0 01*	*0 07*	*0 03*
	Hypercholesterolaemics without clinical atherosclerosis	7	40 1	363	363	3 1	308	326	346	1 61	3 08	2.39	0 13	1 03	0 48
			3 4	*39*	*36*	*37*	*3*	*19*	*18*	*0 13*	*0.39*	*0 52*	*0 03*	*0 25*	*0 12*
	Hypercholesterolaemics with clinical therosclerosis	18	50.8	329	331	3 7	*03	3_3	320	1 8	3.38	_59	0.09	0.82	0.40
			5	*11*	*1*	*14*	*7*	*8*	*10*	*0 14*	*0 90*	*0 33*	*0 07*	*0 08*	*0 10*
	Hyperlipaemics without clinical therosclerosis	6	47.5	400	416	409	345	381	377	4.00	6 62	5 68	0.33	1.39	)
			2.9	*27*	*24*	*0*	*22*	*22*	*15*	*1 10*	*1 39*	*1 06*	*0 04*	*0 72*	—
	Hyperlipaemics with clinical atherosclerosis	4	62.5	379	374	373	347	340	361	3.99	5.51	5 75	0 23	0 90	1 13
			2 9	*63*	*68*	*63*	*50*	*63*	*59*	*0 63*	*1 10*	*1_3*	*0 09*	*0.22*	*0 45*
	Normocholesterolaemic normoglyceridaemic atherosclerotics	0	54	262	*70	*64	_39	286	275	1 30	66	1 81	0 08	0 31	0. 4
			3 6	*5*	*9*	*10*	*8*	*12*	*8*	*0 13*	*28*	*0.23*	*0.01*	*0 10*	*0 05*
Female	Controls	*0	41 6	83	283	271	270	303	296	1 03	1 75	1.27	0 06	0 44	0.26
			3 1	*13*	*14*	*12*	*12*	*12*	*11*	*0 10*	*0 15*	*0 13*	*0 004*	*0 07*	*0.03*
	Hypercholesterolaemics without linical therosclerosis	11	47 1	394	412	393	369	400	398	1 48	2 28	1 67	0 08	0 67	0 34
			3 8	*23*	*1*	*20*	*16*	*17*	*16*	*0 11*	*0.31*	*0 15*	*0 03*	*0 15*	*0 05*
	Hypercholesterolaemics with clinical therosclerosis	8	61.3	346	3 9	344	323	332	353	1 4	_66	_77	0 08	0 43	0 51
			2 3	*1*	*14*	*11*	*14*	*9*	*11*	*0 1*	*0 32*	*0 54*	*0 01*	*0.03*	*0 12*
	Hyperlipaemics without clinical therosclerosis	4	51.5	364	363	357	360	381	382	3 08	4.36	4 18	0 17	0 90	0.67
			9	*56*	*57*	*54*	*43*	*45*	*34*	*0.56*	*1 11*	*0 75*	*0 02*	*0.27*	*0 10*
	Hyperlipaemics with linical therosclerosis	4	6 0	403	417	435	408	439	506	5.32	8.53	10 87	0 18	1 76	2.03
			1.8	*67*	*73*	*64*	*37*	*36*	*42*	*1.02*	*1 43*	*77*	*0 04*	*0 66*	*1.30*

S, S and S = serum before 4 and 7 hours after the fat meal.
Figures in italics are standard errors of the mean.
) 1 S turbidity value is lacking n = 5 yields the following turbidity values 0 _3 + 0 05 0 72 ± 0 06 and 1 10 ± 0 38

jects) in which the cholesterol phospholipid and glyceride-glycerol levels and turbidity of serum were determined immediately before as well as 4 and 7 hours after the fat meal. (See Table 6.) With few exceptions the fasting and 4-hour values of these patients are included in the $S-S_4$ Series. The group classification and results of serum lipid and turbidity estimations are shown in Table 6. Grouping was done according to the same principles as before. The controls were so chosen with respect to age as to make the decades from 20 to 60 years more or less equally numerous. Six men and women were over 60 years of age. The cholesterol level of fasting serum exceeded 300 mg % in 3 male and in no less than 8 female controls.

3. Serum and its subnatant S_4 and S (subnatant after centrifugation at 25000 *g* for 1 hour) were analyzed for glyceride-glycerol content and turbidity in 166 cases. These subjects have been classified by groups in Table 7.

4. Serum and its subnatant, S_7, where serum 7 hours after the fat meal and its subnatant were analyzed in 3 cases as under 3. (See Table 7.)

Comment

It should be borne in mind that the control groups were not composed wholly of normal subjects. Two subgroups can be distinguished namely "healthy subject" and patients with sciatica. These subgroups exhibited significantly unequal serum glyceride-glycerol levels, a fact worth drawing attention to because initially sciatica was accepted as irrelevant in regard to serum lipids. CARLSON (1960) similarly accepted cervical spondylosis, varicose veins, haemorrhoids and inguinal hernia. The mean serum glyceride-glycerol level for his normal men of all age groups (age range 26—73 years) was 1.19 mMol/l, a value practically identical to the writer's 1.14 mMol/l for all the 4 male controls. The corresponding cholesterol and phospholipid levels of fasting serum — 48 and 55 mg % versus 30 and 251 mg % respectively — also agreed closely. Notably however CARLSON's and the writer's control groups had significantly dissimilar age distributions, the latter showing a predominance of the higher age groups (over 45 years) so that the mean age of all the male controls was 50.9 years as against 44.6 years for CARLSON's normal men. But there was no significant difference between the present controls over and under 50 years of age in either the cholesterol phospholipid and glyceride-glycerol levels or the turbidity of fasting serum.

The similarity with respect to all lipid factors between the writer's and CARLSON's control groups is worth stressing because SVANBORG & SVENNERHOLM (1961) as well as CRAMÉR (1962) reported lower cholesterol and phospholipid levels and CRAMÉR had a lower glyceride-glycerol level as well. Conversely MALMCRONA (1960) obtained cholesterol values of more nearly the same order as the writer's. Though genetic and dietetic differences between the populations on the West and East coasts of Sweden (SVANBORG

Group	Sex		Glyceride-glycerol mMol/l			Turbidity		
			I	II	I–II	I	II	I–II
0 hours								
Controls	♂	36	1 14±0 08	1 07±0 07	0 08±0 03	0.09±0 01	0 06±0 00	0 03±0 01
	♀	34	1 0*±0 06	0 99±0 06	0 03±0 01	0 07±0.01	0 03±0.00	0.0 ±0.00
Hypercholesterolaemics without clinical atherosclerosis	♂	8	1 40±0 15	1.51±0 16	−0.03±0 04	0 13±0 05	0.09±0 04	0 04±0.02
	♀	23	1.35±0 09	1.33±0 08	0 0 ±0 0*	0 09±0 01	0 06±0 00	0 03±0 01
Hypercholesterolaemics w th clinical therosclerosis	♂	18	1 71±0.14	1 66±0 13	0 05±0 03	0 09±0 01	0 07±0 00	0 0 ±0 00
	♀	10	1 81±0 _2	1 79±0._1	0 02±0 04	0 09±0 01	0 06±0 00	0 03±0 01
Hyperl paemics without linical therosclerosis	♂	0	4 18±0 87	3 41±0 44	0 78±0 39	0 33±0 09	0 13±0 0	0 *0±0 08
	♀	6	_97±0 43	91±0 43	0.07±0.26	0 15±0 0*	0 10±0 01	0 03±0 0*
Hyperlipaemics with linical therosclerosis	♂	0	3 96±0 68	3 15±0 30	0 81±0 66	0 17±0 01	0 11±0.01	0 06±0.01
	♀	6	4 18±1.3	3 64±0 93	0 34±0 41	0.23±0 09	0 11±0 01	0 12±0 06
Normocholesterolaemic normoglyceridaemic atherosclerotics		9	1 61±0 13	1 67±0 17	−0 07±0 02	0 08±0 04	0 06±0 00	0 0*±0 00
4 hours								
Controls	♂	36	_03±0 14	1 46±0 09	0 37±0 08	0.5*±0 04	0 11±0 01	0 41±0 04
		34	1 60±0 10	1.34±0 09	0.27±0 03	0 41±0 03	0 11±0 01	0.30±0 03
Hypercholesterolaemics without clinical atherosclerosis	♂	8	_43±0 30	1.89±0.21	0 55±0 10	0 84±0 15	0 13±0 02	0 71±0 13
	♀	23	2 18±0 14	1 9±0 01	0.39±0 06	0.53±0 10	0 14±0 0*	0.39±0 09
Hypercholesterolaemics with linical therosclerosis	♂	18	2.01±0_3	.31±0.14	0 70±0.14	0 69±0 11	0 13±0 01	0.57±0 11
	♀	10	2.38±0.32	_*9±0.26	0.30±0 11	0 48±0 07	0 11±0 01	0 38±0 0
Hyperlipaemics without clinical atherosclerosis	♂	0	6 81±0 61	4 11±0.27	0±0 46	2.47±0 51	0 20±0 0*	2.*7±0 50
	♀	6	4 17±0 9	3 74±0 74	0 43±0 *7	1 05±0.30	0 19±0 04	0 83±0.28
Hyperlipaemics with clinical therosclerosis	♂	9	61 ±1 04	3.73±0 40	2.3*±0 3	1 33±0 *9	0._4±0 05	1 09±0.27
		6	3 73±0 67	4 *0±0.57	1.32±0 23	1 67±0 4	0 17±0 0*	1 60±0 40
Normocholesterolaemic normoglyceridaemic therosclerotics	♂	8	3 72±0 42	_49±0.34	1 22±0.18	1._1±0 37	0 13±0 0*	1 06±0 33
hours								
Controls	♂	1	1 _0±0 24	1 31±0.22	0 19±0 06	0.20±0 04	0 07±0 01	0 13±0 03
	♀	9	1 11±0 17	0 94±0 14	0 1 ±0.00	[illegible]	[illegible]	[illegible]
Hypercholest[illegible]								

& SVENNERHOLM, 1961) have been proposed as factors responsible for these discrepancies, they might also be due simply to different criteria for the selection of subjects. The writer's observations seem to support the latter view

The groups of hypercholesterolaemics, hyperlipaemics and normoglyceridaemic normocholesterolaemics with atherosclerosis are rather diffusely demarcated The fact that classification into these groups often was based on serial estimations explains why the hyperlipaemics included patients with substantially normal lipid levels of fasting serum on the day of the fat tolerance test With the present classification some patients would perhaps be included among the hyperlipaemics because their serum lipids for some months exhibited a hyperlipaemic pattern, whereupon several years with entirely normal serum lipids might elapse before another such period begins (cf GROOVER, 1960)

Nor are the groups with and without atherosclerosis distinctly demarcated except that the latter had a significantly lower mean age The frequency of such procedures as say angiography must obviously be low in the symptomless group and high in the group with atherosclerotic manifestations. The difficulty of classifying cases is illustrated by the following example A man of 28 without cardiac distress and a normal Ecg during exercise was subjected to coronary angiography owing to several cases of coronary disease in the family The angiograms disclosed total occlusion of the right coronary artery and atherosclerosis like changes in the left coronary artery

mmediately before nd 4 and 7 hours fter the fat meal

Group	Sex	n	Glyceride-glycerol mM 1/1 I	II	I−II	Turbid ty I	II	I−II
0 hours								
Controls	♂	36	1 14±0 08	1 07±0.07	0 08±0 03	0 09±0 01	0 06±0 00	0 03±0 01
	♀	34	1 02±0 06	0 99±0 06	0 03±0 01	0 07±0 01	0 03±0 00	0.02±0 00
Hypercholesterolaemics without clinical therosclerosis	♂	8	1 49±0 15	1.51±0 16	−0 03±0 04	0 13±0 05	0 09±0 04	0 04±0 02
	♀	22	1.35±0 00	1.33±0 08	0 0 ±0 02	0 09±0 01	0 06±0.00	0 03±0 01
Hypercholesterolaemics with clinical atherosclerosis	♂	18	1 1±0 14	1 66±0 12	0 03±0 03	0 09±0 01	0 07±0 00	0 02±0 00
	♀	10	1 81±0.22	1 79±0_1	0 03±0.04	0 09±0 01	0 06±0 00	0 03±0 01
Hyperlipaemics without clinical therosclerosis	♂	0	4 18±0 67	3 41±0 44	0 78±0 39	0 33±0 09	0 19±0 02	0 ⁹0±0 08
	♀	6	9⁻±0 45	2.91±0 43	0 07±0.26	0 15±0 02	0 10±0 01	0 03±0 02
Hyperlipaemics w th clinical atherosclerosis	♂	9	3 96±0 68	3.15±0.30	0 81±0 66	0 17±0 01	0 11±0 01	0 06±0 01
	♀	6	4 18±1.33	3 64±0 93	0.54±0.41	0 23±0 09	0 11±0.01	0 12±0 06
Normocholesterolaemic normo glyceridaemi therosclerotics	♂	8	1 61±0.13	1 67±0 17	−0 0 ±0 0⁹	0 06±0 04	0 06±0 00	0 0⁹±0 00
4 hours								
Control	♂	36	03±0.14	1 46±0 09	0.57±0.08	0 6 ±0 04	0 11±0 01	0 41±0 04
	♀	34	1 60±0 10	1 34±0 09	0.27±0 03	0 41±0 03	0 11±0 01	0 30±0 03
Hypercholesterolaemics w thout linical therosclerosis	♂	8	2 43±0.30	1 69±0.21	0.35±0 10	0 64±0 15	0 12±0 02	0 1±0 15
	♀	22	_18±0 14	1 9±0 01	0.39±0 06	0.53±0.10	0.14±0 02	0.39±0 09
Hypercholesterolaemics with linical therosclerosis	♂	18	3 01±0.25	2.31±0 14	0 70±0 14	0 69±0.11	0 13±0 01	0.57±0 11
	♀	10	.58±0 32	2.29±0_6	0 30±0 11	0 48±0 07	0 11±0.01	0.38±0 07
Hyperl paemics without linical therosclerosis	♂	9	6 81±0 61	4 11±0.27	_ 0±0 46	47±0.31	0.20±0 0⁹	_27±0.50
	♀	6	4 17±0 79	3 74±0 74	0 42±0 2⁻	1 05±0.30	0.19±0 04	0.85±0_8
Hyperlipaemics with clinical therosclerosis	♂	9	6 12±1 04	3 73±0 40	.37±0. 3	1 33±0.20	0.24±0 05	1 09±0 27
		6	3 73±0 67	4.50±0.5⁻	1.3 ±0 23	1 67±0 4_	0 17±0 02	1.50±0 40
Normocholesterolaemic normo glyceridaemic therosclerotics	♂	8	2.72±0 4	2.49±0.34	1.2⁹±0 18	1 21±0 37	0 13±0 0	1 06±0 33
7 hours								
Controls	♂	1	1.30±0 4	1.31±0 23	0 19±0 06	0 20±0 04	0 07±0 01	0.13±0 03
	♀	0	1 11±0 17	0 94±0.14	0 1 ±0 06	0 22±0 03	0 0⁻±0 004	0 15±0 02
Hypercholesterolaemics with d		1	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

Glyceride-glycerol determ nation The method described by CARLSON & WADSTRÖM (1959) was used in the early stages of the present investigation and CARLSON's (1959) modification thereof in its later stages.

For every series of glyceride-glycerol determinations, a special conversion factor was computed from the results on at least 4 standard tripalmitin references and 4 blanks.

Every cholesterol phospholipid and glyceride-glycerol determination was duplicated on the extract and every series of determinations included standard references Analytical errors of the methods are presented in Table 8

The fat tolerance test Before breakfast after 1 hours fasting, the patient was given a standard meal of 200 ml of cream (40% butter fat) Blood samples for estimation of lipids were drawn immediately before and 4 hours after — in a minority of patients also 7 hours after — ingestion of the cream. In the meantime the in-patient had the freedom of the ward. The out patient was requested to keep reasonably still in the morning before the fat tolerance test and while it was in progress During this period the patients were not allowed to eat and should refrain from smoking and drinking

The blood samples were at first drawn into oxalated tubes (2 mg sodium oxalate per ml.) But as pointed out by SPERRY & SCHÖNHEIMER (1935) volume for volume the lipid levels of oxalated plasma are some 15 per cent lower than those of serum or heparinized plasma as a consequence of altered plasma volume Against this background the following check was made The differences between serum and plasma in turbidity and cholesterol phospholipid and glyceride-glycerol contents were determined in 15 fasting blood samples and 15 four hours after a standard fat meal. The cholesterol, phospholipid and glyceride-glycerol levels of serum turned out to be on average 0 7 per cent (range: 0—+29 %) higher than the corresponding plasma levels, regardless of whether the samples were fasting or postalimentary The mean lipid levels of serum and their ranges were 277 mg % (162—40* mg) cho-

TABLE 8. *Analytical error of the methods*

		Number of duplicates	Range	Mean	Analytical error in per cent
Cholesterol mg%*	serum¹)	50	149—409	304	1.81
	extract	100	214—573	311	0.88
Phospholipids mg%	serum²)	50	175—443	293	2.44
	extract	100	183—433	293	2.19
Glyceride glycerol mMol/l	serum²)	100	0.43—8.56	1.39	2.72
	extract	100	0.43—8.56	1.39	2.13

) single determinations on duplicate extracts of serum.

²) duplicate determinations on duplicate extracts of serum.

CHAPTER IV

METHODS

Turbidity determination The turbidities of serum and plasma were determined as optical density at 650 mμ 10 mm pathway and room temperature in a Beckman B Spectrophotometer using a distilled water blank The wavelength 650 mμ was used for the turbidity measurements in the present investigation because it has been adopted by the majority of previous authors (BLOCK BARKER & MANN 1951 HERZSTEIN 1953 WOLDOW CHAPMAN & EVANS 1954 BRONTE-STEWART & BLACKBURN 1958 KUO & JOYNER 1955 etc) A serum sample whose turbidity exceeded 1 50 units was diluted with 4 volumes of physiological saline 1 50 turbidity units being the upper limit beyond which the dilution curve for chylomicrons ceases to be linear or nearly so

Extraction of lipids (after SVANBORG & SVENNERHOLM 1958) 1 ml serum was pipetted into a 25 ml Erlenmeyer flask containing 15 ml chloroform methanol (1 1 by volume) After shaking the mixture was filtered into a graduated cylinder through a paper boiled in alcohol. The flask was rinsed twice with 3 ml chloroform methanol and the filter paper cone with 3 ml chloroform methanol and then once with 3 ml pure chloroform To remove contaminants phase partition was achieved by adding 3—5 ml physiological saline shaking the cylinder violently for 1 minute and then letting it stand for 24 hours The upper layer was discarded and the lower layer as well as the cylinder walls were washed twice with 3 ml of cleansing fluid (the upper phase formed after mixing 150 ml chloroform, 120 ml methanol and 50 ml physiological saline) After discarding the upper layer pure chloroform was added to the lower up to the 25 ml mark. The resultant extract was used for lipid determination

Cholesterol determination Cholesterol was determined by a modification of the method given by THEORELL (1926 1931) This method has been submitted to extensive comparison with the SPERRY & WEBB (1950) procedure At all serum levels the present method gave 4 8—4 9 per cent higher values (HOOD & ANGERVALL, 1957 1959 CRAMÉR & ISAKSSON 1959) In 50 duplicate determinations there was no significant difference between the mean cholesterol content of the chloroform methanol extract and that of the acetone-ethanol extract Whenever both extractants were used in the present investigation the two cholesterol values were averaged.

Phospholipid determination Serum phospholipids were estimated according to SVANBORG & SVENNERHOLM (1961)

Glyceride-glycerol determination. The method described by CARLSON & WADSTRÖM (1959) was used in the early stages of the present investigation and CARLSON's (1963) modification thereof in its later stages.

For every series of glyceride-glycerol determinations, a special conversion factor was computed from the results on at least 4 standard tripalmitin references and 4 blanks.

Every cholesterol phospholipid and glyceride-glycerol determination was duplicated on the extract and every series of determinations included standard references. Analytical errors of the methods are presented in Table 8.

The fat tolerance test. Before breakfast, after 1 hours fasting the patient was given a standard meal of 200 ml of cream (40 % butter fat). Blood samples for estimation of lipids were drawn immediately before and 4 hours after — in a majority of patients also 7 hours after — ingestion of the cream. In the meantime the in-patient had the freedom of the ward. The out-patient was requested to keep reasonably still in the morning before the fat tolerance test and while it was in progress. During this period the patients were not allowed to eat and should refrain from smoking and drinking.

The blood samples were at first drawn into oxalated tubes (mg sodium oxalate per ml.). But as pointed out by SPERRY & SCHOENHEIMER (1935) volume for volume the lipid levels of oxalated plasma are some 15 per cent lower than those of serum or heparinized plasma as a consequence of altered plasma volume. Against this background, the following check was made. The differences between serum and plasma in turbidity and cholesterol phospholipid and glyceride-glycerol contents were determined in 15 fasting blood samples and 15 four hours after a standard fat meal. The cholesterol, phospholipid and glyceride-glycerol levels of serum turned out to be on average 9.7 per cent (range 0—+20 %) higher than the corresponding plasma levels, regardless of whether the samples were fasting or postalimentary. The mean lipid levels of serum and their ranges were: 277 mg% (16 —402 mg %) cho-

TABLE 8. *Analytical error of the methods*

		Number of duplicates	Range	Mean	Analytical error in per cent
Cholesterol mg%	serum¹)	60	140—400	204	1.61
	extract	100	214—373	311	0.66
Phospholipids mg%	serum¹)	60	173—482	[illegible]	2.44
	extract	100	183—443	300	2.19
Glyceride-glycerol mMol/l	serum²)	100	0.43—3.50	1.05	2.72
	extract	100	0.43—3.54	1.05	3.18

¹) single determinations on duplicate extracts of serum.

²) duplicate determinations on duplicate extracts of serum.

lesterol 285 mg% (174—413 mg%) phospholipids and 1 88 mMol/l (0 52—5 02 mMol/l) glyceride-glycerol In order to render them comparable with the corresponding serum level plasma values for cholesterol phospholipids and glyceride-glycerol obtained in the first part of the series were adjusted to 110 per cent of the value actually estimated

The turbidity estimations showed a much greater range of variation Thus the serum turbidity averaged 0 20 units and ranged from 0 05 to 1 35 units The serum turbidity was on average 0 6 per cent higher than the corresponding plasma turbidity (range —34%—+44%) On inspection oxalated plasma samples are occasionally *found to contain minute particles which* can be made to precipitate by centrifugation at high speeds Calcium oxalate might well contribute to the turbidity of oxalated plasma No correction was applied to the turbidity values of plasma

Following centrifugation of each blood sample at 1000 g for 20 minutes the serum supernatant was pipetted off and its turbidity and cholesterol *phospholipid and glyceride-glycerol* levels determined These serum samples *from a proportion of the patients were* thereupon recentrifuged at 25 000 g for 60 minutes in a Phyve-Pirouette water cooled High Speed Centrifuge Type 804 The serum subnatant thus obtained was aspirated and subjected to similar determinations

In 10 cases the values of the lipid factors 4 hours after ingestion of the *fat meal were estimated in two separate* blood samples Thus after centrifugation of each such sample at 1000 g for 20 minutes on two occasions, the cholesterol phospholipid and glyceride-glycerol levels and turbidity of its serum supernatant were determined, *whereupon each such serum portion* was centrifuged at 25 000 g for 60 minutes on two occasions and the corresponding lipid factors were estimated in its serum subnatant The results of these analyses are presented in Table 9 Except for turbidity the errors of serum centrifugation *at 25 000 g* were smaller than at 1000 g Hence the chylomicron phase is well defined

TABLE 0 *Errors of serum centrifugation at 1000 g and 25 000 g*

	Centrifugal force in g	Range	Mean	Analytical error
Cholesterol mg%	1 000	143—397	239	3 3
	25 000	141—380	229	6
Phospholipid mg%	1 000	164—303	262	4
	25 000	158—380	25[illegible]	3
Glyceride glycerol mMol/l	1 000	0 98 —4.59	[illegible]07	4
	25 000	0 80 —[illegible].59	1.5	4 4
Turbidity	1 000	0 063—1.8	0.552	[illegible]
	25 000	0 045—0.20	0.088	[illegible] 3

DUPLICATE FAT TOLERANCE TESTS

Duplicate fat tolerance tests, with determinations of cholesterol, phospholipids, glyceride-glycerol and turbidity in serum before and 4 hours after ingestion of the fat meal were performed on 57 subjects.

No systematic difference could be demonstrated between the 1st and 2nd fat tolerance tests. Neither the cholesterol, phospholipid and glyceride-glycerol levels nor the turbidity of serum differed significantly on the two occasions. Accordingly the two f t tolerance tests were assumed to be made under similar conditions.

Table 10 presents means (± standard errors) for the lipid levels and turbidity of serum on th two occasions and standard deviations in per cent of the difference in the respective lipid and turbidity between the two occasion. The table reveals that the standard deviation of the difference in fasting serum varied between 10 and 11 per cent of the mean for cholesterol, 12 and 13 per cent for phospholipids, 1 and 3 per cent for glyceride-glycerol and 64 and 271 per cent for turbidity. The great turbidity dispersion i f sting serum from hyperlipaemics w caused by a single excessive value f 3 . If this value is disregarded, the spread w ll be 69 . per cent of the group of hyperlipaemics and 8 . per cent for the whole group.

The associations between the 4-hour values and the corresponding 0-hour values were studied by plotting the respective differences. The strength of the associations was then studied with the aid of χ^2 analysis (Cf. footnote p. 34.)

Turbidity. No significant) association could be demonstrated within any of the groups between the differences in 4-hour and fasting values on the two

) . per cent level.

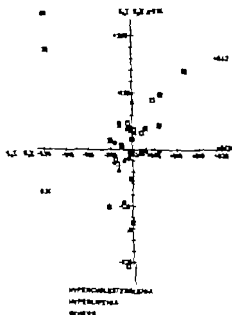


Fig. 2. Association between differences of turbidity in sera 4 hours after ($S_4I - S_4II$) and before ($S_0I - S_0II$) duplicate standard fat meals.

lesterol 285 mg% (174—413 mg%) phospholipids and 1 88 mMol/l (0 52—5 9 mMol/l) glycende-glycerol In order to render them comparable with the corresponding serum level plasma values for cholesterol phospholipids and glyceride-glycerol obtained in the first part of the series were adjusted to 110 per cent of the value actually estimated

The turbidity estimations showed a much greater range of variation Thus the serum turbidity averaged 0 20 units and ranged from 0 03 to 1 35 units The serum turbidity was on average 0 6 per cent higher than the corresponding plasma turbidity (range −34%—+44%) On inspection oxalated plasma samples are occasionally found to contain minute particles which can be made to precipitate by centrifugation at high speeds Calcium oxalate might well contribute to the turbidity of oxalated plasma No correction was applied to the turbidity values of plasma

Following centrifugation of each blood sample at *1000 g* for *20* minutes the serum supernatant was pipetted off and its turbidity and cholesterol *phospholipid and glyceride-glycerol* levels determined These serum samples from a proportion of the patients were thereupon recentrifuged at 25 000 *g* for 60 minutes in a Phyve-Pirouette water *cooled High Speed Centrifuge* Type 804 The serum subnatant thus obtained was *aspirated and subjected to* similar determinations

In 10 cases the values of the lipid factors 4 hours after ingestion of the fat meal were estimated in two separate blood samples Thus after centrifugation of each such sample at 1000 *g* for 20 minutes on two occasions, the cholesterol phospholipid and glyceride-glycerol levels and turbidity of its serum supernatant were determined whereupon each such serum portion was centrifuged *at 25 000 g for 60* minutes on two occasions and the corre*sponding lipid factors were estimated* in its serum subnatant The results of these analyses are presented in Table 9 Except for turbidity the errors of serum centrifugation at 25 000 *g* were *smaller than at 1000 g* Hence the chylomicron phase is well defined

TABLE 9 *Errors of serum centrifugation at 1000 g and 25 000 g*

	Centrifugal force in *g*	Range	Mean	Analytical error in
Cholesterol mg%	1 000	145—307	239	3.3
	25 000	141—380	229	–.6
Phospholipid mg%	1 000	164—393	263	4
	25 000	158—380	252	2
Glyceride-glycerol mM 1/1	1 000	0 98 —4.59	2 07	4 8
	25 000	0.80 —2.59	1.52	4 4
Turbidity	1 000	0.065—1 87	0.55	—
	25 000	0.045—0.20	0.088	40.3

occasions. A positive and significant association emerged when the hypercholesterolaemics and the others were combined into a single group or the entire series was studied (See fig $\chi^2 > 3.84$). Thus, for a single individual the fasting and 4-hour turbidities of serum at two fat tolerance tests are associated in the sense that a higher fasting turbidity on the second occasion often is followed by a higher 4-hour value

Glyceride-Glycerol Differences in 4 hour and fasting values between two fat tolerance tests are more strongly associated than those for turbidity the association being positive and significant for the hyperlipaemics and the hypercholesterolaemics as well as for the series as a whole (See fig 2) Table 11 presents the results of duplicate fat tolerance tests on 8 hyperlipaemics in whom the glyceride-glycerol difference in fasting serum exceeded 1 mMol/l Notwithstanding the considerable spread of the fasting values the 4 hour value was so closely related to the 0-hour value that their ratios were closely similar in 7 of the 8 patients

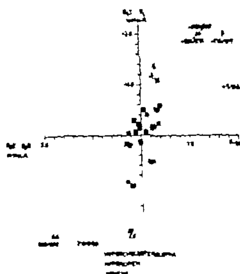


Fig 2 Association between differences of glyceride-glycerol in sera 4 hours after (S_4I − S_4II) and before (S_0I − S_0II) duplicate standard fat meals.

The mean ratio of the 4-hour and fasting serum glyceride-glycerol levels (Cf Chapter VII) was 1 79 for the 30 hypercholesterolaemics, 1 66 for the 1 hyperlipaemics and 1 74 for the 10 others the standard deviations of the difference between the two determinations being 0 37 0 41 and 0 22

TABLE 11 Glyceride glycerol ratio (S_4/S_0) in hyperlipaemics whose S_0 value at duplicate tests exceeds 1 mMol/l

Initials and age	S_0 I	S_0 II	S_4 I	S_4 II	S_4/S_0 I	S_4/S_0 II
KO 23	9 84	18 71	9 93	18 83	1.01	1 01
LA 37	3 63	5.40	6 97	8.87	1 92	1 63
AF 43	[illegible]93	2 78	10 14	2 96	1 13	1.07
HL 53	8 83	8 13	12 93	8.90	1 46	1 33
YB 33	4 84	1.84	9 23	4 21	1 91	2 17
EA 34	10 67	8 81	8 84	7 90	0 83	1 43
JL 38	3 43	4 38	4.83	6 98	1 41	1.57
KJ 65	2 41	4.89	3 26	6.83	1.35	1 51

TABLE 10 *Dupl cate fat tolerance test* (*FTT I and II*)

	Serum lipids before the fat meal				Serum lipids 4 hours after the fat meal			
	Cholesterol mg%	Phosphol pids mg%	Glyceride-glycerol mMol/l	Turbidity	Cholesterol mg%	Phospholipids mg%	Glyceride-glycerol mM l/l	Turbidity
Hyperchol st laemia n = 30								
FTT I	320 ± 11	79 ± 9	1.38 ± 0 10	0 088 ± 0 006	330 ± 13	323 ± 11	–.64 ± 0 13	0 88 ± 0 11
FTT II	329 ± 11	304 ± 9	1 43 ± 0 13	0 094 ± 0 01	332 ± 11	3–8 ± 10	2. 6 ± 0.20	0 08 ± 0 04
diff I – II	33 6	36.1	0 340	0 071	31 4	34.3	0 7"3	0.789
diff in % of FTT mean	10 8	1..	1 3	80.2	0.3	10 6	"0 3	84 9
Hyperl pa mia = 17								
FTT I	342 ± 25	328 ± 1	4 64 ± 0.75	0.281 ± 0.03"	343 ± 23	349 ± 19	6 63 ± 0 73	2.27 ± 0.36
FTT II	351 ± 23	318 ± 15	4 43 ± 0 94	0 438 ± 0 44	342 ± .3	349 ± 20	6.52 ± 0.23	1 80 ± 0.30
diff I – II	37 1	40..	3.348	0.869	40 4	63 2	3.736	1 103
diff in % of FTT mean	10 6	1 .3	3.0	"33	11 8	18.1	36 6	54 4
Others n = 10								
FTT I	211 ± 10	224 ± 10	1 64 ± 0 16	0 093 ± 0.014	"21 ± 11	249 ± 0	2 67 ± 0 31	0.83 ± 0 18
FTT II	210 ± 12	18 ± 14	1 48 ± 0 16	0 083 ± 0 006	"23 ± 14	"3" ± 16	–.59 ± 0.37	1 19 ± 0 37
diff I – II	1	44 3	0 33	0 061	3 3	55..	0.561	0 755
diff in % of FTT mean	10..	19 9	1 1	62.9	14 7	.2.	21 0	80.3
Total = 57								
FTT I	312 ± 11	"93 ± 0	–.51 ± 0 29	0 147 ± 0 03	319 ± 11	318 ± 9	3.33 ± 0 34	0 28 ± 0 13
FTT II	316 ± 11	"93 ± 8	3.33 ± 0 33	0.201 ± 0 03	316 ± 11	318 ± 10	3.85 ± 0.38	1.27 ± 0 14
d ff I – II	33 8	39 0	1 814	0 473	34 0	47 7	2.077	0 874
d ff f FTT mea	10 8	13 3	7 6	"1	10.	13 0	34	68.0

TABLE 12. *Serum cholesterol changes at duplicate fat tolerance tests*

			Cholesterol mg per cent				
			S_0		Cholesterol change		Difference
			A lower	B higher	A	B	A−B
Hyper cholesterolaemia		30	215.2±10.6	242.8±10.7	+8.0±2.6*)	+2.1±3.0	+5.9±4.7
	)	14	214.3±20.0	261.6±19.2	+15.9±5.1*)	−1.4±5.4	+17.4±7.3*)
Hyperlipaemia		17	231.4±22.4	262.3±24.5	+6.9±3.9	−15.4±8.2	+22.3±10.1*)
	*)	13	244.4±26.9	282.1±28.6	+4.2±4.7	−23.2±8.9*)	+27.4±12.8*)
Others		10	207.0±10.0	222.0±11.5	+7.1±17.2	+6.1±2.7*)	+1.0±5.5
	)	2	217.0±9.0	260.5±7.5	−3.5±4.5	+7.0±3.0	−10.5±7.5
Total		57	201.0±10.5	227.6±11.4	+7.6±2.4*)	−2.4±3.1	+10.0±4.1*)
	)	29	231.9±16.3	262.8±16.5	+9.3±3.4*)	−10.6±3.1*)	+19.9±6.7*)

) Subjects with fasting serum cholesterol difference exceeding 20 mg%.

*) = significant difference

between the groups with lower (A) and higher (B) fasting cholesterol levels was not significant. The glyceride-glycerol ratios in these two groups were not unequal (1.77 and 1.73 respectively).

Phospholipids When the phospholipid differences between fasting sera and between 4-hours sera for two fat tolerance tests are plotted it becomes apparent that these differences seem negatively correlated. (Fig. 5.) This is also shown by Table 13 in which the lower phospholipid level of fasting serum is called A and the higher B. Table 13 discloses that the mean phospholipid change 4 hours after a fat meal was significantly greater for Group A than for Group B in the hypercholesterolaemics. The hyperlipaemics and others exhibited similar tendencies. In addition the difference between the phospholipid changes was

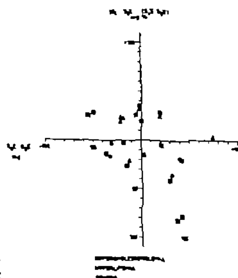


Fig. 5. Association between differences in serum phospholipid changes between 4 and 0 hours [(S_4I − S_0I) − (S_4II − S_0II)] and phospholipid differences of fasting sera (S_0I − S_0II) at duplicate standard fat meals.

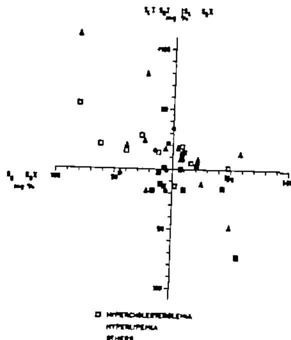


Fig 4 Association between differences in serum cholesterol changes between 4 and 0 hours $[(S_4\text{I} - S\text{ I}) - (S\text{ II} - S\text{ II})]$ and cholesterol differences of fasting sera $(S_0\text{I} - S_0\text{II})$ at duplicate standard fat meals.

respectively The corresponding figures for the series as a whole were 1 74 and 0 36 In other words if this ratio is regarded as a measure of the effect of the fat load its "error of method" would be 0 30 or around 20 per cent The coefficients of correlation between the glyceride glycerol ratios at the 1st and 2nd fat tolerance tests were 0 87 and 0 83 for respectively the hyper cholesterolaemics and the hyperlipaemics

Cholesterol In the present context it is appropriate to call the difference between the cholesterol levels of fasting serum and 4 hour serum the cholesterol change

Differences between the two fasting cholesterol levels as well as between the two 4 hour cholesterol levels have been plotted in Fig 4 which discloses that the correlation between fasting and 4 hour values is negative

When the pairs of fasting cholesterol values were arranged with the lower in one group (A) and the higher in another group (B) it appeared that the sera with the lower cholesterol values showed the greater mean cholesterol change 4 hours after the fat meal (Table 12) The difference in cholesterol change between Groups A and B is significant for the hyper lipaemics and for the hyperlipaemics plus hypercholesterolaemics and for the whole group ($n = 57$)

When only patients with cholesterol differences between the fasting sera of more than 20 mg% were taken into consideration the result was as shown in Table 12 The cholesterol change in A now became significantly greater than that in B for the hyperlipaemics the hypercholesterolaemics and the group as a whole This suggests that a hypercholesterolaemic or hyperlipaemic patient s serum cholesterol change in response to fat loading depends mainly upon the cholesterol level of his fasting serum at the time of the fat meal. The cholesterol change will be largest if his fasting cholesterol level is near his own lower limit

No significant association could be demonstrated between the cholesterol change and the glyceride glycerol ratio ($\chi^2 < 3$ 86)

In all patient categories the mean glyceride-glycerol was lower in the group of fasting sera with the lower cholesterol level although the differenc

TABLE 14

	Association between glyceride-glycerol and		
	Cholesterol	Phospholipids	Turbidit
Hypercholesterolaemia = 20	sign.	sign.	sign.
Hyperlipaemia = 17	—	sign.	sign.
"Others" = 10	—	—	—

be accompanied by a lower glyceride-glycerol value

The significant associations between turbidity and glyceride-glycerol are surprising especially in the hypercholesterolaemic group with its small turbidity differences (mean 0 04) The correlation coefficients c for the hypercholesterolaemics, hyperlipaemics and others and for the series as a whole were 0 47 0 87 0 60 and 0 62 respectively These findings illustrate the value of turbidity measurements as a test for neutral fat changes in the individual subjects.

In 4-hour sera the only association studied was that between turbidity and glyceride-glycerol and this association was not significant for any of the subgroups, nor for the series as a whole ($n = 57$) The association between turbidity and glyceride-glycerol in the hylomicron phase was studied in 43 cases and proved to be significant for the complete series as well as for hypercholesterolaemics ($n = 23$) and hyperlipaemics ($n = 1$) The glyceride-glycerol content of the chylomicron phase and that of serum exhibited a significant positive correlation.

Comment The present investigation demonstrated that the 4 hour and fasting differences in serum turbidity and glyceride-glycerol level at duplicate fat tolerance tests are significantly correlated

A number of authors have expressed the opinion that duplicate fat tolerance tests yield fairly similar results, most of them merely having compared the curves for serum turbidity or total serum lipids after repeated fat meals (MORETON 1950 MESSINGER & POROSOWSKA, 1953 BARRITT 1956 OSMON ZINN & WHARTON 1957 BRONTE STEWARD & BLACKBURN 1958) The 4-hour and fasting turbidity and glyceride-glycerol differences observed in the present investigation varied considerably at duplicate fat tolerance tests, suggesting that analysis of association is a much better measure of variability than curve tracing.

The results thus imply that *the higher the subject's fasting serum glyceride-glycerol level or turbidity the* higher his values 4 hours after a fat meal. This result could be due to more rapid fat absorption, but there exists no evidence indicating that the rate of fat absorption is accelerated when the serum neutral fat level is enhanced. *The* reason might be reduced fat removal from the blood stream.

TABLP 13 *Serumphospholipid changes at d plicate fat tolera ce tests*

	Phospholipids in mg per cent				
	S		Phospholipid change		Difference
	A lower	B higher	A	B	A−B
Hyper cholesterolaemia −30	285.2± 9 0	315 6± 8 4	+3⌒0±6.2*)	+17 6±3 8*)	+15.1± 3.3*)
Hyperlipaemia n=17	207.2±14 8	337 9±16 9	+34 1±0 6*)	+18.9±31 6	+13.1±13 0
Others n=10	203.8±11 5	237 6±10 0	+27.5±7 7*)	+16.6±5 1)	+10 ±10.
Total	277 5± 8.2	308.6± 8.2	+32.2±4.0*)	+17.8±3 4*)	+14.3± 3.2*)

*) = significant difference

also significant for the series as a whole Nothing new emerged when the analysis was based on duplicate fat tolerance tests at which the phos pholipid difference of fasting serum exceeded 20 mg%

No significant association could be demonstrated within subjects between the serum phospholipid change and the serum glyceride-glycerol ratio

Whereas the hypercholesterolaemics and the series as a whole exhibited a significantly higher mean glyceride glycerol content *of those sera that had* the higher mean phospholipid level the hyperlipaemics and others merely displayed similar tendencies Groups A and B had similar mean glyceride glycerol ratios

Association between the glyceride-glycerol level of fasting serum and its cholesterol phospholipids and turbidity In the individual subject the re spective differences in fasting sera were studied by χ^2 analysis[1]) The results are set out in Table 14

The fact that the groups were so small might be responsible for the non significant associations However if the hyperlipaemics and "others are combined into a single group all its associations will be significant Hence it seems justified to assume that in the individual subject there exist as sociations between cholesterol or phos pholipids and glyceride-glycerol in the sense that a lower value of cholesterol *or phospholipids can be expected to*

[1]) Both vari bles of the observations re put int co-ord nat system and both riables are divided to two equal groups. The result ing 4 groups can be described as $\frac{a \mid b}{\ \ d}$ where $+b=d+$ and $a+d=b+$ is calc lated

$$\chi^2 = \frac{(ac-bd)^2 (\ +b+\ +d)}{(\ +b)(\ +d)(\ +\ )(b+d)}$$

and the coefficient of association ( ) is calc lated

$$r^2 = \frac{\chi^2}{(\ +b+\ +d)}$$

CHAPTER VI

FREQUENCY ANALYSES OF CHANGES IN SERUM LIPID FACTORS AT THE FAT TOLERANCE TEST

In order to select relationships it might be interesting to analyze, a pilot study was made with respect to the frequencies of changes in the turbidity as well as glyceride-glycerol, cholesterol and phospholipid levels of serum after the fat meal. This frequency study was performed on the $S_4 - S - S$ Series (Table 15) which was divided into three main groups: (A) controls, (B) hypercholesterolaemics and hyperlipaemics without and (C) all patients with clinical atherosclerosis. Within each of these groups the frequency of rises after 4 and 7 hours as well as the frequency of decreases from the 4th to the 7th hour were calculated for each lipid factor (Table 15)

Four hours after the fat meal the turbidity and the glyceride-glycerol level of serum had risen in 100 per cent of all groups. The corresponding percentages for cholesterol rises ranged from 61 to 71 per cent. The phospholipid level rose in 100 per cent of Group A, 93 per cent of Group B and 91 per cent of Group C. Accordingly it is of interest to investigate the changes in turbidity glyceride-glycerol and phospholipids 4 hours after the fat meal

While the serum turbidity was higher 7 hours after the fat meal than before it in practically 100 per cent of all three groups, the corresponding proportions for serum glyceride-glycerol increases ranged from 68 to 81 per

TABLE 15. *Frequency (in %) of relative changes in turbidity glyceride-glycerol cholesterol and phospholipids in serum immediately before and 4 and 7 hours after the fat meal*

	$S_4 > S$			$S_7 > S_4$			$S_7 > S$		
	A	B	C	A	B	C	A	B	C
Turbidity	100	100	100	93	98	100	77	81	89
Glyceride-glycerol	100	100	100	89	79	81	93	71	74
Cholesterol	61	71	69	27	61	63	73	54	44
Phospholipids	100	93	91	93	93	88	80	50	47

S, S_4 och S_7 = Serum immediately before and 4 and 7 hours after the fat meal.

A = Controls = 44,

B = Hypercholesterolaemics and hyperlipaemics without clinical atherosclerosis = 28,

C = Normocholesterolaemics, hypercholesterolaemics and hyperlipaemics with clinical atherosclerosis = 43

Most workers have reported no or negligible serum cholesterol alterations in response to a fat load (BLOOR, 1916 HILLER *et al* 1924 BLIX 1925 HIRSCH & CARBONARO 1950 POMERANZE BEINFIELD & CHESSIN 1954 ALBRINK & MAN 1956) The present observation that *serum cholesterol* alterations after fat loading depend upon whether *the individual s fasting* cholesterol lies high or low in his own *range of serum cholesterol variation* implies that one should not expect any *simple reproducibility of the serum* cholesterol change in response to duplicate *fat tolerance tests* while the serum cholesterol level of some persons rises that of others falls

It is known that after a fat meal the serum phospholipid rise (MAN & GILDEA 1932 POMERANZE BEINFIELD & CHESSIN 1954 HAVEL 1957 etc) and this is borne out by the results of the present investigation In analogy with the case of cholesterol in serum it holds true for the individual subject that the lower the initial serum phospholipid level the higher it rises after a fat meal The fasting serum with the lower phospholipid level also exhibits the lower glyceride glycerol content *Conversely the lower fasting* glyceride-glycerol value showed the smaller increment *4 hours after the* fat meal Supposing that the absorption *from the digestive tract is similar* at the duplicate fat tolerance tests *this would mean that the removal of* neutral fats from the blood was more rapid *in Group A* According to HAVEL (1957) the phospholipid content rises in the *chylomicrons* and in the very low density lipoproteins during the lipaemic *phase and in the high density* lipoproteins during the postlipaemic *phase The latter rise could conceivably* take place more rapidly during a high *rate of removal of neutral fats from* the blood and this could result in a *higher total phospholipid increase* 4 hours after a fat meal

When the *individual subject s* diet on the two occasions for the duplicate *fat tolerance tests* was unchanged, changes in the fasting serum levels of *glyceride-glycerol cholesterol* phospholipids and turbidity of serum usually were *in the same direction With* respect to glyceride-glycerol and turbidity this seems to mean that equilibrium obtains between visible fats (chylomicrons and very low density lipoproteins capable of producing turbidity) and *invisible fats*

It was true of the whole series that *the higher the glyceride-glycerol level* of 4-hour serum the greater the chylomicron phase of such serum *Significant* associations existed between the turbidity *and the glyceride-glycerol* content of the chylomicron phase of 4 *hour serum as well as between the* glyceride glycerol content of the chylo*micron phase of 4 hour serum* and the turbidity of such serum But the association *did not extend so far as to* include the turbidity and the glyceride *glycerol* level of 4 hour serum Consequently it may be said that turbidity differences between 4-hour sera in duplicate fat tolerance tests are not a measure of changes in the glyceride glycerol content of such sera.

CHAPTER VII

THE FAT TOLERANCE TEST IN VARIOUS GROUPS OF SUBJECTS

Serum lipids and turbidity in the control groups

The key role played by the control groups in these analyses justifies some preliminary comments on these group

Means and standard deviations for the glyceride-glycerol level and turbidity of serum immediately before as well as 4 and 7 hours after the fat meal in the controls are given in Table 16 The glyceride-glycerol level as well as the turbidity are skewly distributed (cf Fig 6) Conversion of these values into logarithms yields a distribution which is accepted as normal. Antilogarithms for the dispersion (mean $\pm 2s$) are given in the table (i.e. 95 % ranges) The mean glyceride-glycerol levels and turbidities of male and female controls do not differ significantly The fact that the glyceride-glycerol values tended to rise before the age of 50 years in the control group as a whole suggests that age might be a factor here So the glyceride-glycerol was studied in age-matched groups ($n = 27$) of male and female controls but no significant difference emerged

In fasting serum positive and significant correlations exist between phospholipids and glyceride-glycerol as well as between turbidity and glyceride-glycerol but apparently not between cholesterol and glyceride-glycerol nor between either cholesterol or phospholipids and turbidity

TABLE 16. *Glyceride-glycerol [and] turbidity in serum of controls 0 4 and 7 hours after the fat meal*

	Glyceride-glycerol mMol/l		Turbidity	
	Mean	95% range	Mean	95% range
Men				
0 hrs } n = 41	1.14	0.[illegible]–2.06	0.10	0.03–0.24
4 hrs }	2.01	0.[illegible]–4.11	0.43	0.16–1.14
7 hrs n = 24	1.37	0.47–3.23	0.21	0.04–0.60
Women				
0 hrs } n = 38	1.00	0.40–1.91	0.09	0.03–0.18
4 hrs }	1.87	0.[illegible]–3.11	0.42	0.13–1.00
7 hrs n = 20	1.27	0.44–2.40	0.26	0.07–0.69

cent The serum cholesterol level was lower 7 hours after than before the fat meal in approximately 75 per cent of Group A No corresponding changes in serum cholesterol could be demonstrated in Groups B and C (39 and 47%) The serum phospholipids were higher 7 hours after than before the fat meal in from 88 to 98 per cent of Groups A B and C Accordingly it is of interest to study the changes in serum turbidity glyceride-glycerol *cholesterol and phospholipids 7 hours* after the fat meal

In 77 81 and 60 per cent of Groups A B and C the serum turbidity fell significantly from the 4th to the 7th hour after the fat meal These frequencies were not significantly unequal At 7 hours the serum turbidity was higher than at 4 hours in 3 of the 24 male and 7 of the 20 female controls i e in 23 per cent of Group A Concomitantly 2 men and no woman showed a higher serum glyceride-glycerol level The glyceride-glycerol level fell from the 4th to the 7th hour after a fat meal in 95 per cent of Group A The corresponding proportions of Groups B and C were significantly lower (71 and 74%) From the 4th to the 7th hour after the fat meal a serum cholesterol reduction was noted in 73 per cent of Group A as against 54 per cent of Group B and 44 *per cent of Group C This proportion of* the controls (Group A) significantly exceeds that of the atherosclerotic subjects (Group C)

The serum phospholipids decreased in 60 per cent of Group A (significant) but *showed only random variations in* Groups B and C The frequency in Group A was significantly higher than that in Group C

TABLE 17 *Distribution of serum glyceride-glycerol before (x) and 4 hrs after the fat meal (y) in relation to regression line for the controls $\hat{y} = 1.55x$. The observed frequency over regression line compared with the expected frequency (50%)*

Group	Number				
	under	over	total	sign. on 5% level	c[1])
Men					
Hypercholesterolaemics without clinical atherosclerosis	4	7	11	no	+0.27
Hypercholesterolaemics with clinical atherosclerosis	10	18	28	no	+0.18
Hyperlipaemics without clinical atherosclerosis	8	6	14	no	+0.97
Hyperlipaemics with clinical atherosclerosis	5	3	8	no	–0.89
Normocholesterolaemics normoglyceridaemics atherosclerotics	2	11	13	yes	+0.33
Women					
H perpercholesterolemmes without clinical atherosclerosis	13	13	26	no	+0.38
Hypercholesterolaemics with clinical atherosclerosis	9	9	18	no	+0.33
Hyperlipaemics without clinical therosclerosis	6	0	6	yes	+1.00
H perlipaemics with clinical therosclerosis	4	4	8	no	+1.00

1) = correlation between serum glyceride-glycerol before and 4 hours after the fat meal estimated according t $\sqrt{\chi^2}$

The table reveals that this association f tted the regression equation $\hat{y} = 1.55x$ in all but two of the groups. When 95 per cent confidence limits are estimated allowance must be made *partly* for the fact that the variance of y was not constant and *partly* for the non normal d stribution of the y values about the line of regression. On the assumption that the logarithms of all y's corresponding to a *given* x are normally distributed the estimated limiting functions bounding the area where 95 per cent of the population falls will be (cf Fig 6).

$$\hat{y} = 1\,11x$$

$$\hat{y} = 2.10x$$

Serum lipids 4 hours after and immediately before the fat meal

Glyceride-glycerol Preliminary observations (ANGERVALL, 1959) suggested that the fat meal caused the serum *glyceride-glycerol to rise more the* higher the glyceride glycerol level immediately before the fat meal In an individual subject moreover duplicate fat tolerance tests disclosed an association between the serum glyceride-glycerol levels 4 hours after and immediately before the fat meal (p 20)

The nature of the association between the serum glyceride glycerol levels immediately before (S_0) and 4 hours after (S_4) the fat meal — in the controls was studied by evaluating the expression

$$S_4 = \alpha + \beta S_0.$$

A linear relationship is assumed between the S_4 and S_0 values The relationship can be written

$$\bar{y} = \alpha + \beta x$$

where α and β are constants $\bar{y}$ is the *conditional mean value of S_4 and x is* the value of S_0. α and β are estimated in the usual manner (cf SNEDECOR, p 108) The hypotheses $\alpha = 0$ and $\beta = 0$ are tested If the hypothesis $\alpha = 0$ is accepted then the ratio of y and x is an estimate of the relationship between S_4 and S_0. *If the hypothesis $\beta = 0$ is* rejected by a test at the 5 per cent level the relationship is considered significant The test is performed as a t test where $t = \frac{b}{s_b}$ (b is an estimate of β and s_b the standard deviation of b)

In order to compare two *lines e g* the test of the hypotheses $\alpha_1 = \alpha_2$ and $\beta_1 = \beta_2$ the analysis has been performed as an analysis of covariance (as described by SNEDECOR, p 395) For men *the estimate of the coefficient of x* was

$$b_1 = 1\ 69$$

and for women it was

$$b_2 = 1\ 60$$

But as $(b_1 - b_2)$ did not deviate significantly from zero (i e there was no *statistically significant difference in* the S_4 S_0 ratio between men and women) the estimate

$$b = 1\ 65$$

was found for the entire control group Because $b = 1\ 65 \neq 0$ the association between S_4 *and S_0 is significant (on* the 5% level)

Considering that the glyceride glycerol values could not be regarded as having a normal distribution the control group was compared with the other groups according to the following principles. The number of observations falling above and below the regression line $\bar{y} = 1\ 65x$ *were calculated for* each group whereupon the observed frequency above the line was compared with the expected frequency of 50 per cent If no difference significant on the 5 per cent level could be demonstrated the hypothesis was accepted that the *group under consideration* exhibited the same association between S_4 and S_0 as the control group The results of this analysis are presented in Table 17

TABLE 17 *Distribution of serum glyceride-glycerol before (x) and 4 hrs after the fat meal (y) in relation to regression line for the controls $\hat{y} = 1.65x$. The observed frequency over regression line compared with the expected frequency (50%)*

Group	Number				
	under	over	total	sign. on 5% level	r[1])
Men					
Hypercholesterolaemics without clinical atherosclerosis	4	7	11	no	+0.27
Hypercholesterolaemics with clinical atherosclerosis	10	18	28	no	+0.18
Hyperlipaemics without clinical atherosclerosis	8	6	14	no	+0.97
Hyperlipaemics with clinical atherosclerosis	5	3	8	no	+0.80
Normocholesterolaemic normo-glyceridaemic atherosclerotics	2	11	13	yes	+0.28
Women					
Hypercholesterolaemics without clinical atherosclerosis	13	13	26	no	+0.39
Hypercholesterolaemics with clinical atherosclerosis	9	9	18	no	+0.33
Hyperlipaemics without clinical atherosclerosis	6	0	6	yes	+1.00
Hyperlipaemics with clinical atherosclerosis	4	4	8	no	+1.00

[1]) = correlation between serum glyceride-glycerol before and 4 hours after the fat meal estimated according to $\sqrt{r^2}$

The table reveals that this association fitted the regression equation $\hat{y} = 1.65x$ in all but two of the groups. When 95 per cent confidence limits are estimated allowance must be made *partly* for the fact that the variance of y was not constant and *partly* for the non-normal distribution of the y values about the line of regression. On the assumption that the logarithms of all y's corresponding to a given x are normally distributed, the estimated limiting functions bounding the area where 95 per cent of the population falls will be (cf. Fig. 6):

$$\hat{y} = 1.11x$$

$$\hat{y} = 2.46x$$

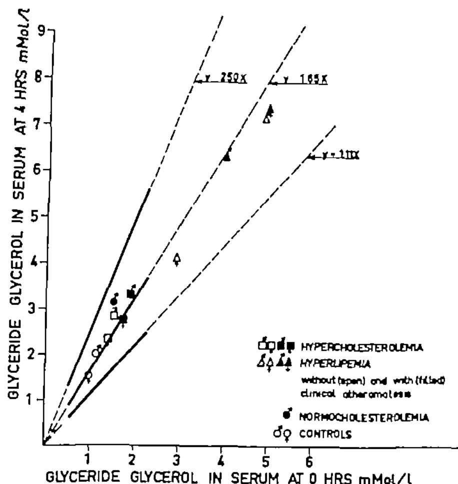


Fig 6 Relation between glyceride-glycerol in serum 4 hours after and before the f t meal Heavy lines represent the regression for controls and its 95 per cent confidence lim ts.

The individual variation is consider able the relative variation about the regression line averages approximately 20 per cent in other words the error of a value y_x estimated from the line may be up to ± 20 per cent of $\hat{y}_x$.

With the possible exception of two groups — "male normoglyceridaemic normocholesterolaemics with atherosclerosis" and "female hyperlipaemics without clinical atherosclerosis" — the glyceride glycerol ratio (S_4 S_0) did not deviate significantly from that for the controls in any group

When among all subjects with hyper lipaemia one selects those whose gly ceride-glycerol level of fasting serum (S_0) exceeds 4 mMol/l one finds that this subgroup has a glyceride glycerol ratio lying significantly below the line $\hat{y} = 1\ 65x$ This suggests that the re lation $\hat{y} = 1\ 65x$ is valid *only* up to a limiting S_0 value of some 4 mMol/l whereupon the line presumably curves towards the abscissa

As a supplement to the foregoing analyses, the frequency of extreme values was studied. The results are

TABLE 18. *The frequency of extremes of the quotient between glyceride-glycerol in serum 4 hrs after (y) and before (x) the fat meal*

Sex	Group	y/x		
		<1.11*)	1.11–2.80	>2.80*)
Males	Controls	1	39	2
	Hypercholesterolaemics without clinical atherosclerosis	0	9	2
	Hypercholesterolaemics with clinical atherosclerosis	1	26	1
	Hyperlipaemics without clinical atherosclerosis	1	11	3
	Hyperlipaemics with clinical atherosclerosis	0		1
	Normoglyceridaemic, normocholesterolaemic atherosclerotics	0	10	3
Females	Controls	0	36	0
	Hypercholesterolaemics without clinical atherosclerosis	1	23	2
	Hypercholesterolaemics with clinical atherosclerosis	3	13	0
	Hyperlipaemics without clinical atherosclerosis	0	6	0
	Hyperlipaemics with clinical atherosclerosis	1	8	0

*) The range 1.11–2.80 corresponds to the reconverted mean ± 2 of the regression of log y upon because of unsymmetrical distribution of y about the regression line $\hat{y} = 1.63x$

presented in Table 18. The table reveals that 4-hour serum had a glyceride-glycerol level exceeding 2.80 times that of fasting serum in 12 of 117 males and in of 93 females. The difference between men and women is significant ($\chi^2 > 3.84$) even after elimination of the group of normoglyceridaemic normocholesterolaemics with atherosclerosis (wherein males only are represented).

Summing up it may be remarked that the glyceride-glycerol level of serum 4 hours after the fat meal is similarly related to that immediately before it in all groups with and without clinical atherosclerosis, perhaps with the exception of normoglyceridaemic normocholesterolaemic men with atherosclerosis, hyperlipaemic women without clinical atherosclerosis and

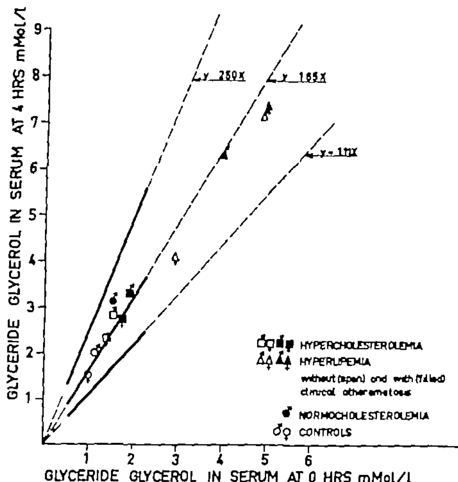


Fig 0 Relation between glyceride-glycerol in serum 4 hours after and before the fat meal Heavy lines represent the regression for control and its 95 per cent confidence limit

The individual variation is considerable the relative variation about the regression line averages approximately 20 per cent in other words the error of a value y estimated from the line may be up to ± 20 per cent of $\hat{y}_x$.

With the possible exception of two groups — male normoglyceridaemic normocholesterolaemics with atherosclerosis" and "female hyperlipaemics without clinical atherosclerosis" — the glyceride-glycerol ratio (S_4 S_0) did not deviate significantly from that for the controls in any group

When among all subjects with hyperlipaemia one selects those whose glyceride glycerol level of fasting serum (S_0) exceeds 4 mMol/l one finds that this subgroup has a glyceride-glycerol ratio lying significantly below the line $\hat{y} = 1.65x$ This suggests that the relation $\hat{y} = 1.65x$ is valid only up to a limiting S_0 value of some 4 mMol/l whereupon the line presumably curves towards the abscissa.

As a supplement to the foregoing analyses the frequency of extreme values was studied The results are

TABLE 1 *The frequency of extremes of the quotient between glyceride-glycerol of serum 4 hrs after (y) and before (x) the fat meal*

Sex	Group	y		
		<1.11	1.11–[illegible]	>[illegible]
Male	Controls	1	[illegible]	
	Hypercholesterolaemics without clinical atherosclerosis	[illegible]	9	
	Hypercholesterolaemics with clinical atherosclerosis	1	[illegible]	1
	Hyperlipaemics without clinical atherosclerosis	1	11	2
	Hyperlipaemics with clinical atherosclerosis	0		1
	Normoglyceridaemic normocholesterolaemic atherosclerotics	0	10	3
Female	Controls	[illegible]	34	
	Hypercholesterolaemics without clinical atherosclerosis	1	23	2
	Hypercholesterolaemics with clinical atherosclerosis	3	1	[illegible]
	Hyperlipaemics without clinical atherosclerosis	[illegible]	6	0
	Hyperlipaemics with clinical atherosclerosis	1	[illegible]	0

) The range 1.11 2.40 corresponds to the reconverted mean ± 2 of the regression of log y upon because of unsymmetrical distribution of y about the regression line $\hat{y} = 1.63x$

presented in Table 18. The table reveals that 4-hour serum had a glyceride-glycerol level exceeding 2.40 times that of fasting serum in 1 of 117 males and in of 95 females. The difference between men and women is significant ($\chi^2 > 3.84$) even after elimination of the group of normoglyceridaemic normocholesterolaemics with atherosclerosis (wherein males only are represented).

Summing up it may be remarked that the glyceride-glycerol level of serum 4 hours after the fat meal is similarly related to that immediately before it in all groups with and without clinical atherosclerosis perhaps with the exception of normoglyceridaemic normocholesterolaemic men with atherosclerosis hyperlipaemic women without clinical atherosclerosis and

those hyperlipaemics whose fasting serum had a glyceride-glycerol level of at least 4 mMol/l Moreover in the series as a whole the frequency of extremely high (compared with the fasting value) serum glyceride-glycerol levels *4 hours after the fat meal* was higher among men than among women

Turbidity No correlation could be demonstrated in any group between the turbidities of sera immediately before and 4 hours after the fat meal (Comparison between turbidities of different groups see p 53 and Table 23

Cholesterol and phospholipids Table 2 p 17 presents cholesterol levels of fasting serum and cholesterol changes 4 hours after the fat meal In 9 of 11 groups the cholesterol levels on the latter occasion tended to be higher than on the former All groups include a majority of subjects with cholesterol rises the frequency of rises *in the entire series being 63 5 per cent* ($n = 212$) which significantly exceeds the 50 per cent expected from random variation As a rule however the cholesterol changes fell within the range *of the standard error of the method* and the mean cholesterol change for the controls exhibited no correlation to either age stature or body surface area

Serum phospholipid rises 4 hours after the fat meal were the rule The mean rises in the various groups are presented in Table 2 p 17 which shows that the mean phospholipid change was positive and *lay between 17 and 30* mg% The mean phospholipid change was significant for all groups No difference could be demonstrated between men and women or between subjects with and without *clinical atherosclerosis*. No correlation could be detected in any group between the serum phospholipid level immediately before and 4 hours after the fat meal A posi*tive association* was demonstrated between absolute phospholipid rises and relative glyceride-glycerol rises after the fat meal in the control and hypercholesterolaemic groups but not in the groups of hyperlipaemics and normoglyceridaemic normocholesterolaemics with clinical atherosclerosis This correlation is significant for both men and women and men plus women among the controls and hypercholesterolaemics

Serum lipids 7 hours after and immediately before the fat meal

Glyceride-glycerol The regression of the serum glyceride-glycerol level 7 hours after the fat meal (y) upon that *immediately before it* (x) for male (1) and female (2) controls had the following equations

(1) $\hat{y} = 1\ 13x + 0\ 03 \quad n = 24$
$s_b = 0\ 1\ \ 1 \quad s_{y.x} = 0\ 400 \quad r = 0\ 80$

(2) $\hat{y} = 1\ \ 5x - 0\ 04 \quad n = \ 0$
$s_b = 0\ 117 \quad s_{y.\ } = 0\ 325 \quad r = 0\ 92$

As no significant difference was present between the regression lines the corresponding *regression* was analyzed in the control group as a whole the equation being

$$\hat{y} = 1\ 1\ x + 0\ 01 \quad n = 44$$
$$s_b = 0\ 102 \quad s_{y.x} = 0\ 310 \quad r = 0\ 8$$

The observations were normally distributed about the regression line The constant in the equa*tion is not signi*

ficantly different from zero The coefficient of regression differs significantly from zero but not from unity implying that the association between the serum glyceride-glycerol levels hours after and immediately before the fat meal was statistically significant Thus generally speaking in the controls the serum glyceride-glycerol level 7 hours after the fat meal was substantially equal to that immediately before it

Data on the occurrence of positive extremes in the various groups are presented in Table 19 revealing that 7 of 9 groups exhibited significantly higher frequencies of extremes than the controls. The exception were the group of hypercholesterolaemic females without clinical atherosclerosis with no extremes and the group of normocholesterolaemic normoglyceridaemic atherosclerotics The hypercholesterolaemic plus hyperlipaemic groups with and without clinical atherosclerosis displayed no significant difference in the frequency of extremes

When the subjects were classified by serum glyceride-glycerol levels immediately before the fat meal up to and over mMol/l and their di tribution about the line $y = 1\ 17x$ was studied it appeared that whereas 4 observations fell above and 42 below the line in the class up to _ mMol/l 21 observations f ll above and 9 below the line in the class over _ mMol/l, the inter-class difference being significant

Analysis of extremes yielded similar results While 0 of 85 observations up

TABLE 19. *Glyceride-glycerol in serum 0 and 7 hours after the fat meal. Comparison between the frequencies of extremes*

Groups	Without clinical atherosclerosis			With clinical atherosclerosis		
	Mean ± 2	> mean + 2	χ^2-test	Mean + 2	> mean − 2	χ^2 test
Controls, men	22[1])	1		–	–	–
Controls, women	20	0	–	–	–	–
Hypercholesterolaemics						
men	4	3	sign.	13	3	sign.
women	11	0		2[1])	3	sign.
Hyperlipaemics						
men	3	3	sign.	2	2	sign.
women	2	2	sign.	0	4	sign.
Normoglyceridaemic normocholesterolaemics	–	–	–	7	2	–

The dispersion ( ) about the regression line for the whole control group $\hat{y} = 1\ 1$ = 0 31 mMol/l extremes = observations more than 2 over the line.

[1]) Includes value more than 2 below the regression line.

TABLE 20 Regression of serum glyceride-glycerol 7 hours after (y) upon that immediately before ( ) th fat meal

Group	Sex		Regression equation	s_b	F	
Controls	♂+♀	44	$\hat{y} = 1\ 17x + 0\ 01$	0 102	0.310	0.8‾
Hypercholesterolaemics without clinical atherosclerosis	♂+♀	18	$\hat{y} = 1\ 96x - 0.95$	0.505	0 7_3	0.68
Hypercholesterolaemics with clinical atherosclerosis	♂+♀	26	$\hat{y} = 0.57x - 0.18$	0 432	1 *93	0.36
Hyperlipaemics without clinical therosclerosis	o+♀	10	$\hat{y} = 0.93x + 1.31$	0 111	0 74	0 94
Hyperlipaemics with clinical therosclerosis	♂+♀	8	$\hat{y} = \ 72x - 4.34$	0.233	1 0 1	0.9
Normocholesterolaemic normo glyceridaemi atherosclerotics	♂+♀	0	$\hat{y} = 1.28x - 0\ 10$	0.323	0 486	0 80
Hypercholesterolaemia	♂+♀	44	$\hat{y} = 1\ 69x - 0\ 46$	0.33	1 104	0 61
Hyperlipaemia	♂+♀	18	$\hat{y} = 1\ 60x - 0.32$	0 258	1 8	0 82
Total	♂+♀	115	$\hat{y} = 1\ 43x + 0\ 04$	0 071	1 166	0.88

to 2 mMol/l fell outside 2 standard deviations 17 of 30 over 2 mMol/l did so These proportions are significantly unequal *In other words if the serum* glyceride-glycerol level immediately before the fat meal exceeds 2 mMol/l the corresponding value 7 hours after the fat meal will be proportionately higher than if the former is lower Within these two classes there was no *difference between subjects with and* without clinical atherosclerosis

Since the regression lines for male and female controls did not deviate significantly corresponding groups of males and females were combined in the subsequent analysis The regression equations for the various groups are presented in Table 20 Statistically the regressions for the hypercholesterol aemic groups with and without clinical atherosclerosis did not deviate so these groups were combined So were the hyperlipaemic groups because they were deemed too small to justify any assessment of biological differences As a result the following equations for the *regression of the serum glyceride gly* cerol level 7 hours after the fat meal (*y*) upon that immediately before it (*x*) were obtained for the hyperchol esterolaemics *(1) hyperlipaemics (*)* and normocholesterolaemic normoglyceridaemic atherosclerotics (3)

(1) $\hat{y} = 1.69x - 0.46$ $n = 44$
$s = 0.332$, $s_{y.x} = 1.104$ $r = 0.61$

(2) $\hat{y} = 1.60x - 0.3[illegible]$ $n = 18$
$s = 0.238$ $s_{y.x} = ..128$ $r = 0.62$

(3) $\hat{y} = 1.22x - 0.16$ $n = 9$
$s = 0.3.3$ $s_{y.x} = 0.486$ $r = 0.90$

None of these regression lines deviates significantly from that for the controls. For the series as a whole the following equation was obtained

$\hat{y} = 1.43x + 0.04$ $n = 115$
$s = 0.071$ $s_{y.x} = 1.166$ $r = 0.86$

where 0.04 does not differ from zero but 1.43 deviates significantly from unity.

Summing up it may be said that the serum glyceride-glycerol level 7 hours after the fat meal is strongly dependent on that immediately before it. For male plus female controls the former can be estimated at 1.17 times the latter. The non-controls exhibited higher frequencies of positive extreme values. With the possible exception of hypercholesterolaemic women no difference was found between subjects with and without clinical atherosclerosis.

Turbidity. No correlation could be demonstrated between turbidities of sera before and 7 hours after the fat meal. See further p. 63 and Table 23.

Cholesterol and phospholipids

Seven hours after the fat meal the serum cholesterol had fallen 8.3±2.8 mg% in the control group as a whole (n = 44), 12.0±3.8 mg% in the female controls (n = 20) and 5.3±3.4 mg% in the male controls (n = 24). The first two differences but not the last are significant. No corresponding differences were found in the other groups.

No association could be demonstrated between the magnitude of the phospholipid rise 7 hours after the fat meal and the phospholipid level of fasting serum. In the series as a whole the phospholipid rise was weakly correlated to the glyceride-glycerol rise (n = 115, r = 0.42). The phospholipid rises in groups with glyceride-glycerol rises up to and over 1 mMol/l were 17.5±1.7 and 48.1±6.0 mg%, and are significantly unequal. The correlation between the phospholipid and glyceride-glycerol rises was significant in the latter (n = 47, r = 0.32) but not in the former group (n = 68, r = 0.19).

Summing up, a phospholipid rise in serum generally persisted 7 hours after the fat meal and was positively associated with the corresponding glyceride-glycerol increase, especially when the latter exceeded 1 mMol/l.

Serum lipids 7 and 4 hours after the fat meal

Glyceride-Glycerol. Preliminary investigations revealed that the turbidity of serum usually fell from the 4th to the 7th hour after the fat meal, suggesting that the removal of lipids from the blood stream exceeded the absorption of lipids from the gastro-intestinal tract. In the light of the close association demonstrated between the serum glyceride-glycerol levels 4 hours after and immediately before the fat meal as well as between those

7 hours after and immediately before the fat meal *one would expect* to find a similar association between the serum glyceride-glycerol levels 7 and 4 hours after the fat meal. *One would* expect the glyceride glycerol difference between the 4th and 7th hour to be *dependent on the value* immediately before the fat meal because $\hat{y}_4 = 1.65x\ S_0$ and $\hat{y}_7 = 1.17x\ S_0$ for the controls *so* $\hat{y}_4 - \hat{y}_7 = S_0(1.65 - 1.17) = 0.48\ S_0$. *No* such equality could be demonstrated *for the controls* ($\chi^2 = 0$, n = 44) however and therefore it seemed interesting *to analyze the association between the serum glyceride-glycerol levels 7 and 4 hours after the fat meal*

As when the association between the *4-hour and 0 hour* values *was determined*, the regression of the 7 hour *glyceride glycerol level (y) upon that 4 hours (x) after the fat meal was found for* male (1) and female (2) controls

(1) $\hat{y} = 0.49x + 0.27$ n = 24
$s_b = 0.110$ $s_{y.x} = 0.480$ r = 0.67

(2) $\hat{y} = 0.91x - 0.14$ n = 20
$s_b = 0.081$ $s_{y.x} = 0.238$ r = 0.91

The regression coefficient for *female controls significantly* exceeds that for male (cf *Fig* 7) and the constants differ from zero. The observations are normally distributed about the regression lines

For the purposes of the subsequent analysis male and female groups and also groups with and without clinical atherosclerosis were kept separate. Since the resulting groups of hyperlipaemics were small they were combined with the corresponding hypercholesterolaemic groups. The regression equations for the various groups thus formed will be found in Table 21 and the regression lines are presented in Fig 7. There was no difference between the male and female groups of hyperlipae-

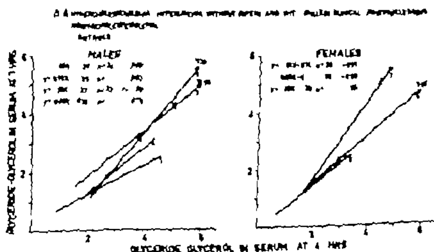


Fig 7. Relation between glyceride glycerol in serum 4 and 7 hours after the fat meal

TABLE 1 *Regression of serum glyceride-glycerol 7 hours after (y) upon that 4 hours after (x) the fat meal*

Group	Sex		Regression equation			r
	♂	4	$\hat{y} = 0.49$ 0.27	0.110	0.490	0.6
Control		26	$\hat{y} = 0.81 - 0.14$	0.091	0.224	0.91
Hypercholesterolaemics +	♂	13	$\hat{y} = 0.79x + 0.20$	0.097	0.858	0.93
Hyperlipaemics		13	$\hat{y} = 0.81x - 0.12$	0.170	0.657	0.85
without clinical atherosclerosis	♂ +	25	$\hat{y} = 0.82x + 0.04$	0.082	0.790	0.93
Hypercholesterolaemics +	♂	22	$\hat{y} = 1.20x - 1.3$	129	0.993	0.90
Hyperlipaemics		12	$\hat{y} = 1.4x - 1.23$	0.134	1.0.5	0.9
with clinical atherosclerosis	♀ +	34	$\hat{y} = 1.42x - 1.78$	0.173	0.736	0.93
Normocholesterolaemic normoglyceridaemic atherosclerotics	♂	9	$\hat{y} = 0.80x$ 0.33	0.21	0.301	0.7

mics plus hypercholesterolaemics without clinical atherosclerosis. But the regression line for the corresponding females with clinical atherosclerosis lay on a significantly higher level than that for the males. Moreover both the latter groups had significantly higher coefficients of regression than the groups without clinical atherosclerosis. The group of normocholesterolaemic, normoglyceridaemic atherosclerotics had a regression line with higher slope than the male controls but lower slope than other males with clinical atherosclerosis and it was on a lower level than that for hypercholesterolaemics plus hyperlipaemics without clinical atherosclerosis.

This means that in two of three comparisons between corresponding male and female groups the latter exhibited relatively slower fat removal from the blood stream and/or delayed absorption between the 4th and 7th hour after the fat meal. This fat removal also seems slower in groups with than in groups without clinical atherosclerosis.

So far it has been assumed that the regression of the 7 hour glyceride-glycerol values upon the 4 hour values is linear. When the glyceride-glycerol values 4 hours after the fat meal for all the subjects ($n = 110$) were classified (see Table 24) it appeared that the regression could be considered linear up to a serum glyceride-glycerol level of approximately 4 mMol/l (a) while the spread was comparatively great for glyceride-glycerol levels exceeding 4 mMol/l (b). The following regression equations were calculated:

(a) $\hat{y} = 0.83x + 0.20$, $n = 03$
$s = 0.078$, $s = 0.857$, $r = 0.83$

(b) $\hat{y} = 1.13x - 0.73$, $n = 22$,
$s = 0.182$, $s = 1.886$, $r = 0.80$

where y and x are the serum glyceride-glycerol level 7 and 4 hours, respectively, after the fat meal. The coefficients of regression differ significantly.

Accordingly in the present subjects the association between the serum glyceride-glycerol level 4 and 7 hours

TABLE 22 Relation between serum glyceride-glycerol 7 (S_7) and 4 (S) hours after the fat meal

	S-classes in mMol/l				
	0–2	–3	3–4	4–6	6
S mean	1.5	2.39	3 43	4.82	8 69
S_7 mean	1 08	1 78	2.33	4 68	[illegible]
$S - S_7$ mean	+ 0 44	+ 0 61	+ 1 10	+ 0 14	– 0.32
$\frac{S - S_7}{S} \times 100$	+29	+26	+32	+ 3	– 6
n	32	41	20	13	9
$S > S_7$ n		5	4	4	4
$\frac{n}{n} \times 100$	0	12	20	24	44

after the fat meal *depended upon* whether the 4 hour serum glyceride glycerol level was high or low the boundary being approximately 4 mMol/l Below this limit the serum glyceride glycerol level fell about 30 per cent from the 4th to the 7th hour after the fat meal and above this limit it fell negligibly or even rose Thus the 7 hour value exceeded the 4 hour value (i e lies above the 45 line in Fig 8) for 11 of 93 subjects in group (a) versus 8 of 22 in group (b) (significant difference) In this respect no difference *could be demonstrated between* subjects without ($n = 28$ not including controls) and with ($n = 43$) clinical atherosclerosis either above or below a 4 hour serum glyceride glycerol level of 4 mMol/l As appears from Table 22 and Fig 8 the frequency of cases with high 7 hour levels in per cent of the class frequency rose from 0 per cent in the class 0–2 mMol/l to some 44 per cent at levels exceeding 6 mMol/l

On the other hand when the distribution about the regression line for the controls ($\bar{y} = 0.66x + 0$ or $n = 44$ $s_b = 0.076$ $s_{y.x} = 0.146$ $r = 0.74$) was studied it appeared that all hyperlipaemics (18 of 18) and 34 of 44 hypercholesterolaemics (14 of 18 without and 20 of 26 with clinical atherosclerosis) fell above it (*significant*) versus only 4 of 9 normocholesterolaemic normoglyceridaemic atherosclerotics The results were similar when this analysis was limited to the range of variation exhibited by the controls Consequently compared with the controls hypercholesterolaemics and hyperlipaemics *regardless of whether or not* they had clinical atherosclerosis exhibited a higher serum glyceride glycerol level 7 hours after the fat meal in proportion to the 4-hour value

In sum then the serum glyceride glycerol levels 7 and 4 hours after the fat meal were significantly associated *This association was not the same in* these subjects at low 4 hour values (up to 4 mMol/l) and at high 4 hour values (over 4 mMol/l) The demonstrated difference between subjects

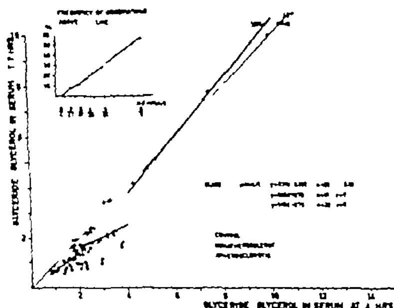


Fig. 4. Relation between glyceride glycerol in serum 4 and 7 hours after the fat meal

with and without clinical atherosclerosis can to a large extent be accounted for by these deviating associations. Regardless of the presence of clinical atherosclerosis hypercholesterolaemics and hyperlipaemics exhibited a higher 7 hour value than the controls in proportion to the 4 hour values.

Cholesterol and phospholipids From the 4th to the 7th hour after the fat meal the serum cholesterol level decreased 8.4±3.1 mg% in the male controls and 13.8±4.9 mg% in the female controls, both these differences being significant. The corresponding decreases in serum phospholipids were 7.3±3.2 mg% in male controls (significant), 6.7±3.6 mg% in female controls and 7.0±2.3 mg% in the control group as a whole (significant). No similar changes were demonstrated in the other groups for either cholesterol or phospholipids.

Comment

Many authors have expressed the view that in fat tolerance tests there is a relationship between atherosclerosis and a prolonged and elevated chylomicron curve (MORETON 1947, 1948, 1950; BECKER, MEYER & NECHELES 1950) or serum turbidity curve (SCHWARTZ, WOLDOW & DUNSMORE, 1954; WOLDOW, CHAPMAN & EVANS 1954; BARRITT 1956; BRONTE-STEWART & BLACKBURN 1958; MITCHELL & BRONTE-STEWART 1959; BOUCHIER & BRONTE-STEWART 1961). HORLICK (1956, 1957) on the other hand felt that a high fat

TABLE 22. *Relation between serum glyceride-glycerol 7 (S_7) and 4 (S) hours after the fat meal*

	S-classes in mMol/l				
	0–	2–3	3–4	4–6	6
S_4 mean	1.52	2.39	3.43	4.8	8.69
S_7 mean	1.08	1.78	2.33	4.66	9.1
$S - S_7$ mean	+ 0.44	+ 0.61	+ 1.10	+ 0.14	− 0.1
$\frac{S - S_7}{S} \times 100$	+29	+26	+32	+ 3	− 6
n	32	41	20	13	9
$S > S_7$	2	5	4	4	4
$\frac{n}{-} \times 100$	0	12	20	24	44

after the fat meal depended upon whether the 4 hour serum glyceride glycerol level was high or low the boundary being approximately 4 mMol/l Below this limit the serum glyceride glycerol level fell about 30 per cent from the 4th to the 7th hour after the fat meal and above this limit it fell negligibly or even rose Thus the 7 hour value exceeded the 4 hour value (i e lies above the 45 line in Fig 8) for 11 of 93 subjects in group (a) versus 8 of 22 in group (b) (significant difference) In this respect no difference could be demonstrated between subjects *without* ($n = 28$ not including controls) and with ($n = 43$) clinical atherosclerosis either above or below a 4 hour serum glyceride-glycerol level of 4 mMol/l As appears from Table 22 and Fig 8 the frequency of cases with high 7 hour levels in per cent of the class frequency rose from 0 per cent in the class 0—2 mMol/l to some 44 per cent at levels exceeding 6 mMol/l

On the other hand when the distribution about the regression line for the controls ($\bar{y} = 0.56x + 0$ or $n = 44$ $s_b = 0.06$ $s_y = 0.146$ $r = 0.4$) was studied it appeared that all hyperlipaemics (18 of 18) and 34 of 44 hypercholesterolaemics (14 of 18 without and 20 of 26 with clinical atherosclerosis) fell above it (significant) versus only 4 of 9 normocholesterolaemic normoglyceridaemic atherosclerotics The results were similar when this analysis was limited to the range of variation exhibited by the controls Consequently compared with the controls hypercholesterolaemics and hyperlipaemics regardless of whether or not they had clinical atherosclerosis, exhibited a higher serum glyceride-glycerol level 7 hours after the fat meal in proportion to the 4 hour value

In sum then the serum glyceride glycerol levels 7 and 4 hours after the fat meal were significantly associated This association was not the same in those subjects at low 4 hour values (up to 4 mMol/l) and at high 4 hour values (over 4 mMol/l) The demonstrated difference between subjects

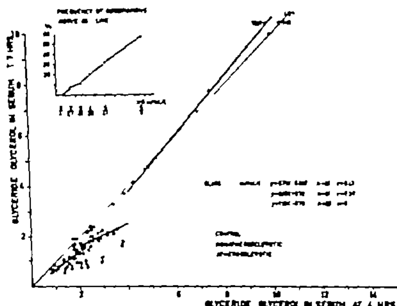


Fig. 8. Relation between glyceride glycerol in serum 4 and hours after the fat meal

with and without clinical atherosclerosis can to a large extent be accounted for by these deviating associations Regardless of the presence of clinical atherosclerosis, hypercholesterolaemics and hyperlipaemics exhibited a higher hour value than the controls in proportion to the 4 hour values.

Cholesterol and phospholipids From the 4th to the 7th hour after the fat meal the serum cholesterol level decreased 8.4±3.1 mg% in the male controls and 13.8±4.9 mg% in the female controls both these differences being significant The corresponding decreases in serum phospholipids were 7.3±2.2 mg% in male controls (significant) 6.7±3.8 mg% in female controls and 7.0±2.3 mg% in the control group as a whole (significant) No similar changes were demonstrated in the other groups for either cholesterol or phospholipid

Comment

Many authors have expressed the view that in fat tolerance tests there is a relationship between atherosclerosis and a prolonged and elevated chylomicron curve (MORETON 194 1948 1950- BECKER, MEYER & NECHELES 1950) or serum turbidity curve (SCHWARTZ, WOLDOW & DUNSMORE, 195 WOLDOW CHAPMAN & EVANS 1934 BARRITT 1956 BRONTE-STEWART & BLACKBURN 1958 MITCHELL & BRONTE STEWART 1959 BOUCHIER & BRONTE STEWART 1961) HORLICK (1956 1957) on the other hand felt that a high-fat

test meal failed to uncover lipid metabolic individuals with atherosclerosis (total fatty acids determination) POMERANZE BEINFIELD & CHESSIN (1954) and KUO JOYNER & REINHOLD (1956) maintained that hypercholesteraemia and hyperlipaemia as such gave rise to prolonged lipaemia (total fatty acids) The present investigation bears out the latter workers Moreover DENBOROUGH (1963) found that the turbidity as well as the triglyceride level of serum after a fat meal were higher in patients with ischaemic heart disease than in controls and this difference was correlated to differences in the triglyceride level of fasting serum The results of the present investigation is in agreement with this finding

The behaviour of the serum cholesterol after a fat meal agrees well with the findings of HIRSCH & CARBONARY (1950) POMERANZE, BEINFIELD & CHESSIN (1954) and ALBRINK & MAN (1956) all of whom reported no or negligible cholesterol changes after a fat meal Others have reported increases of the order of 5 per cent (HILLER et al 1934 BLOTNER 1935 BRUHN 1940 HAVEL, 1957 KINGSBURY MORGAN & SHERVINGTON 1960) No corollary to the association between body weight and the cholesterol changes induced in serum by a fat meal in BLOTNER's (1935) investigation was found in the present study No significant serum cholesterol increase was demonstrated in any group 4 hours after the fat meal Both control groups showed a small but significant cholesterol fall between the 4th and 7th hour usually to a level below the cholesterol concentration in fasting serum In this respect there was significant difference between the controls and the subjects with clinical atherosclerosis. This bears out NIKKILÄ & KONTTINEN (1962) who observed a serum cholesterol decrease 6 hours after a meal of 55 g butter fat

That the serum phospholipids may rise after a fat meal has been reported previously (MAN & GILDEA 1932 POMERANZE, BEINFIELD & CHESSIN 1954 HAVEL, 1957 KINGSBURY MORGAN & SHERVINGTON 1960) ALBRINK & MAN (1956) on the other hand found no such rise after a fairly small test meal (60 g fat) The amount of fat ingested seems a significant factor This is illustrated by the small phospholipid increase recorded 3 hours after a meal of 60 g of fat as contrasted with the 18 per cent rise obtained after 3 5 g to 4 g of fat per kg body weight (MAN & GILDEA 1932) The results reported by POMERANZE BEINFIELD & CHESSIN (1954) TALBOTT & KEATING (1962) and SKIBBE & HAUDE (1963) indicate that the greatest alimentary lipaemia is attended by the highest serum phospholipid increase The latter finding is borne out by the present investigation But like serum cholesterol the phospholipids decreased significantly between the 4th and 7th hours after the fat meal in the controls but not in the other group

As appears from Table 6 page the male controls had significantly

higher turbidity of fasting serum than the female and the other groups exhibited similar tendencies. The corresponding serum glyceride-glycerol levels for males also tended to be higher than for females in all groups. There was a significant difference between the male and female controls in the serum glyceride-glycerol level 4 hours after the fat meal and also between male and female hypercholesterolaemics with clinical atherosclerosis in serum turbidity at 4 hours. Generally speaking therefore in the present series of subjects the serum turbidity and glyceride-glycerol variations in response to the fat meal displayed few significant differences between men and women.

Table 23 presents some direct comparisons between the serum turbidity and glyceride-glycerol values observed within groups immediately before 4 hours after and 7 hours after the fat meal. The table reveals that the group of normocholesterolaemic normoglyceridaemic atherosclerotics (males only) in no respect differed significantly from the male controls (but see also p. 42). Conversely all groups of hypercholesterolaemics (males and females with and without clinical atherosclerosis) exhibited significant differences from the corresponding controls in regard to the serum glyceride-glycerol level immediately before the fat meal but not to all the 4 hour and 7 hour values. Furthermore Student's t was consistently higher for the fasting differences than for the 4-hour and 7 hour differences something characterizing practically all comparisons of this type made. The corresponding analysis of the turbidity variations in response to the fat meal disclosed a significant difference in the turbidity of fasting serum between female hypercholesterolaemics and controls but the differences were on the whole definitely smaller and usually not significant and Student's t was usually consistently smaller than the corresponding values for glyceride-glycerol differences and often not significant when the latter were significant.

Neither the glyceride-glycerol level nor the turbidity of serum observed at fat tolerance tests showed any significant differences between the combined groups with and the combined groups without clinical atherosclerosis.

The group presented in the table comprising 10 men with established myocardial infarction all had shown a clinical course and Ecg changes typical of this affection. The group included 6 subjects classified as hypercholesterolaemics, 1 hyperlipaemic (with a fasting-serum glyceride-glycerol level of 4.44 mMol/l) and 3 normoglyceridaemic normocholesterolaemics. The group of myocardial infarctions differed significantly from the male controls only in the serum glyceride-glycerol level immediately before the fat meal.

The group of subjects with stenosis of the renal artery comprised 9 hypercholesterolaemics (6 males and 3 females) and 3 normo-glyceridaemic normocholesterolaemics. This group differed from the combined control groups with respect to the glyceride-glycerol levels (with the highest values of Stu

TABLE *3 *D // rences between groups in serum glyceride-glycerol and turbidity before 4 and hours after th fat meal*

Sex	Group	n	Glyceride-glycerol mMol/l S_4	S	S_t	Turbidity S	S	S_t
Male	Controls	24	1 18	2.24	1.37	0.08	0.57	0.31
	compared with							
	Normocholesterolaemic normo-glyceridaemic atherosclerotics	9	1 50	2 66	1.81	0.08	0.51	0 34
	t		1.33	1.27	1 40	0 21	0 71	0.53
	Hypercholesterolaemics without clinical atherosclerosis	7	1 61	3 08	2.59	0 13	1 05	0.48
	t		2.	1 94	2.30	1 93	1 88	2.20
	Hypercholesterolaemics with clinical atherosclerosis	18	1 82	3.38	2 58	0 09	0 82	0.40
	t		2 74	3.36	2 94	1 11	1 93	1 63
	Myocardial infarction	10	1 93	2 5*	2 76	0 10	0 72	0.55
	t		*5 12*	*1 64*	*2.04*	*0.89*	*0 03*	*1.29*
Female	Controls	20	1 03	1 78	1 *7	0 06	0 44	0.*8
	compared with							
	Hypercholesterolaemics without clinical therosclerosis	11	1 47	2.38	1 67	0 09	0 67	0.34
	t		2 80	2 44	1 98	3.18	1.3*	1 41
	Hypercholesterol emics with clinical therosclerosis	8	1 4	2.66	.7	0 08	0 42	0 1
	t		3.01	2.66	2 74	2.30	0 23	1 96
Male+female	Control	44	1 12	2 01	1.32	0 07	0 51	0.23
	compared w th							
	S bject with renal artery stenosis	12	1 7	3.04	2 07	0 09	0.53	0.43
	t		3 92	2 76	.10	1 16	0.18	1 82
	Hypercholesterolaemics without linical atherosclerosis	18	1 5	2 65	1 98	0 10	0 8	0.40
	compared with							
	H percholesterolaemics with clinical atherosclerosis	**	1 9	3 10	2.64	0.09	0 0	0 48
	t		1 93	1 63	1 61	−1 74	−0 78	0 6
	Hyperlipaemics thout linical therosclerosis	9	4 17	5.8	5.26	0.21	1.25	0 91
	compared w th							
	Hyperlipaemics with clinical therosclerosis	8	4 06	7 02	8.31	0 20	1.35	1.5*
	t		0.50	0.82	1 63	−0.32	0 13	0 97

dent t for the differences in fasting serum) but not to turbidity.

Summarizing it may be inferred from the foregoing that (a) the serum glyceride-glycerol level is better than its turbidity as a criterion for distinguishing between clinical groups; (b) the t values for the fasting-serum glyceride-glycerol differences are generally higher than those for the 4-hour and 7 hour differences, implying that the degree of statistical significance of the difference between clinical groups produced by fat tolerance tests usually is no higher than that observed between fasting values, and (c) hypercholesterolaemics or hyperlipaemics with and without clinical atherosclerosis do not differ significantly in the serum glyceride-glycerol levels and turbidities either before or 4 and 7 hours after the fat meal.

TABLE 3 *Differences between groups in serum glyceride-glycerol and turbidity before, 4 and 7 hours after the fat meal*

Sex	Group	n	Glyceride-glycerol mMol/l			Turbidity		
			S_0	S_4	S_7	S_0	S_4	S_7
Male	Controls	24	1.18	2.24	1.37	0.06	0.37	0.31
	compared with							
	Normocholesterolaemic, normoglyceridaemic atherosclerotics	9	1.50	2.66	1.81	0.08	0.51	0.34
	t		1.33	1.2	1.40	0 1	0.71	0.53
	Hypercholesterolaemics without clinical atherosclerosis		1.61	3.06	2.59	0.13	1.03	0.45
	t		2.7	1.94	2.30	1.95	1.88	2.20
	Hypercholesterolaemics with clinical atherosclerosis	14	1.82	3.36	2.64	0.00	0.82	0.40
	t		3. 4	3.36	2.84	1.11	1.93	1.63
	Myocardial infarction	10	1.93	3.22	2.76	0.10	0.72	0.53
	t		6.12	1.84	2.04	0.89	0.95	1.39
Female	Control	20	1.05	1.75	1.27	0.06	0.44	0.36
	compared with							
	Hypercholesterolaemics without clinical atherosclerosis	11	1.4	2.38	1.67	0.08	0.67	0.34
	t		2.80	2.44	1.93	2.19	1.37	1.41
	Hypercholesterolaemics with clinical atherosclerosis	8	1. 4	2.46	.77	0.08	0.42	0.41
	t		3.01	3.56	2.74	2.30	0.25	1.96
Male + female	Controls	44	1.12	.01	1.32	0.07	0.51	0.33
	compared with							
	Subjects with renal artery stenosis	1	1.72	2.04	2.07	0.09	0.53	0.43
	t		2.0	2.76	2.10	1.15	0.18	1.62
	Hypercholesterolaemics without clinical atherosclerosis	18	1.34	.03	1.88	0.10	0.82	0.40
	compared with							
	Hypercholesterolaemics with clinical atherosclerosis	36	1. 9	2.10	2.64	0.09	0. 0	0.48
	t		1.93	1.63	1.61	−1.74	−0.78	0.67
	Hyperlipaemics without clinical atherosclerosis	9	4.1	5.62	5. 6	0.21	1. 3	0.91
	compared with							
	Hyperlipaemics with clinical atherosclerosis	8	4.06	0	6.31	0.40	1.25	1.04
	t		0.50	0.82	1.63	−0.32	0.13	0.9

dent *t* for the differences in fasting serum) but not to turbidity

Summarizing it may be inferred from the foregoing that (a) the serum glyceride-glycerol level is better than its turbidity as a criterion for distinguishing between clinical groups; (b) the *t* values for the fasting-serum glyceride-glycerol differences are generally higher than those for the 4 hour and hour differences, implying that the degree of statistical significance of the difference between clinical groups produced by fat tolerance tests usually is no higher than that observed between fasting values; and (c) hypercholesterolaemics or hyperlipaemics with and without clinical atherosclerosis do not differ significantly in the serum glyceride-glycerol levels and turbidities either before or 4 and 7 hours after the fat meal.

TABLE 23 *Differences between groups in serum glycerid -glycerol and turbid ty before 4 nd hours after the fat meal*

Sex	Group	n	Glyceride-glycerol mMol/l			Turbidity		
			S	S	S	S	S	S
Male	Controls	4	1 18	2.24	1.37	0 04	0.5	0_1
	compared witl							
	\ rmocholesterolaemic normo-glyceridaemic atherosclerotics	0	1.50	2.66	1 81	0 08	0.61	0 24
	t		1.33	1.27	1 40	0.21	0 71	0.63
	Hypercholesterolaemics without linical atherosclerosis	7	1 61	3 08	.59	0 13	1 05	0 48
	t		–	1 94	2.30	1 93	1 88	2.20
	Hypercholesterolaemics with li ical atherosclerosis	18	1 62	3.38	2.58	0 09	0.82	0 46
	t		3 74	3.16	2.94	1 11	1.93	1 63
	My ocardi l infarction	10	1 93	3.22	2.76	0 10	0 7	0 52
	t		5 1	1.84	2 04	0.89	0 95	1.30
Female	Controls	20	1 03	1 75	1._7	0.06	0 44	0.26
	compared with							
	Hypercholesterolaemics without clinical therosclerosis	11	1 4	2.38	1 67	0 08	0 67	0.34
	t		2.80	2.44	1 98	2 19	1.37	1 41
	Hypercholesterolaemics with cli ical atherosclerosis	8	1 4	2.66	77	0 08	0 42	0 51
	t		3 01	2.56	74	_.30	0.23	1 90
M le + female	Control	44	1 12	01	1 32	0 07	0 51	0.23
	compared witl							
	Subject with renal rtery stenosis	1	1 7	3.04	_.07	0.00	0 63	0.43
	t		3 02	2 76	10	1 15	0 18	1 6
	Hypercholesterolaemics without clinical therosclerosis	18	1 5	2.65	1 98	0.10	0 82	0 40
	compared with							
	Hypercholesterolaemics with clinical atherosclerosis	*6	1 0	3 10	2.84	0 09	0 0	0 48
	t		1 95	1 63	1 61	−1 74	−0 78	0 67
	Hyperlipaemics without cl cal therosclerosis	9	4 1	5.8	5.26	0.21	1.25	0 91
	compared with							
	Hyperl paem cs with linical atherosclerosis	8	4 66	7 02	5.21	0.20	1 23	1 38
	t		0.50	0.62	1 63	−0.32	0 13	0 07

dent's t for the differences in fasting serum) but not to turbidity

Summarizing it may be inferred from the foregoing that (a) the serum glyceride-glycerol level is better than its turbidity as a criterion for distinguishing between clinical groups; (b) the t values for the fasting-serum glyceride-glycerol differences are generally higher than those for the 4 hour and 7-hour differences implying that the degree of statistical significance of the difference between clinical groups produced by fat tolerance tests usually is no higher than that observed between fasting values and (c) hypercholesterolaemics or hyperlipaemics with and without clinical atherosclerosis do not differ significantly in the serum glyceride-glycerol levels and turbidities either before or 4 and 7 hours after the fat meal.

CHAPTER VIII

GLYCERIDE GLYCEROL LEVEL AND TURBIDITY OF SERUM OF ITS SUBNATANT AND ITS CHYLOMICRON PHASE

The glyceride-glycerol and turbidity of serum its subnatant and its chylomicron phase (after centrifugation at 25000 *g* for 1 hour) immediately before and 4 hours after the fat meal were determined in 166 subjects and in 52 subjects also 7 hours after the fat meal all subjects being on ordinary diet The results are set out in Table 7 p 23

Glyceride glycerol of whole fasting serum and its subnatant

Controls The glyceride-glycerol content of fasting serum differed significantly from that of its subnatant in both male and female controls the respective mean differences being 0 08 ±0 03 and 0 03±0 01 mMol/l These differences which do not deviate significantly represent the glyceride-glycerol of the chylomicron phase of fasting serum

The regressions of the glyceride-glycerol content of the subnatant (y) upon that of whole serum (x) in male (1) and female (2) controls were as follows

(1) $y = 0\ 81x + 0\ 15 \quad n = 36$
$s_b = 0\ 062 \quad s_{y.x} = 0\ 08 \quad r = 0\ 01$

(2) $y = 1\ 01x - 0\ 04 \quad n = 34$
$s_b = 0\ 030 \quad s_{y.x} = 0\ 063 \quad r = 0\ 99$

The constants +0 15 and −0 04 both deviate from zero and the regression coefficient 0 81 is less than unity The lines representing these two equations have different slopes. This suggests that the relationship between the glyceride glycerol level of fasting serum and that of its subnatant was different in male and female controls

Other groups No significant difference between the glycende-glycerol of fasting serum and that of its subnatant could be demonstrated in any of the other groups nor in either the combined groups with or the combined groups without clinical atherosclerosis (cf Table 7) The table reveals that those groups whose fasting serum had a high glyceride-glycerol level had a larger chylomicron phase than those groups whose corresponding glyceride glycerol level was low The controls and the other groups were therefore divided into classes with fasting serum glyceride-glycerol below 1 and between 1 and 2 mMol/l (cf Table ?4) It appeared that unlike the other groups, the controls showed a significant difference between the glycende glycerol levels of whole fasting serum and of its subnatant — the chylomicion phase — in both these classes (0 029±0 004 and 0 043±0 016 mMol/l respectively) Each of these chylomicron phases also significantly exceeded that in the corresponding class of the other groups

TABLE 24 *Glyceride-glycerol of fasting serum and its substrate and their difference (chylomicron phase)*

	Serum glyceride-glycerol in mMol/l				
	0–1	1–	2–3	3–4	4
Controls —	32	26		—	—
Serum	0.74	1.33	[illegible]		
Substrate	0.71	1.28	1.73		
Chylomicron phase	0.03	0.04	0.56		
Standard error of mean	0.004	0.018	0.450		
Chylomicron phase in per cent of serum	5	3	24		
Others —	7	[illegible]	23	6	10
Serum	0.84	1.49	[illegible]	3.40	6.32
Substrate	0.88	1.48	2.36	3.65	4.44
Chylomicron phase	0.019	0.0025	0.083	— 0.[illegible]	1.882
Standard error of mean	0.012	0.015	0.043	0.183	0.610
Chylomicron phase in per cent of serum	2	0	4	— 8	30
Total series —	39	56	23	6	1
Serum	0.78	1.41	[illegible]	3.40	6.32
Substrate	0.74	1.39	2.31	3.65	4.44
Chylomicron phase	0.022	0.017	0.123	— 0.278	1.88
Standard error of mean	0.003	0.011	0.035	0.183	0.610
Chylomicron phase in per cent of serum	3	2	5	— 8	30

Turbidity and glyceride-glycerol of whole fasting serum and its substrate

It appeared that the turbidity and glyceride-glycerol of fasting serum exhibited a positive and significant correlation in the entire series ($n = 166$, $r = 0.79$) as well as in three of its eleven subgroups (Table 25).

The regressions of the turbidity (y) upon the glyceride-glycerol level (x) of fasting serum in the combined groups of hypercholesterolaemics and hyperlipaemics with (1) and without (2) clinical atherosclerosis were (Fig. 9)

(1) $y = 0.082x + 0.029$ $n = 45$
$s = 0.4086$ $s = 0.0084$ $r = 0.90$

(2) $y = 0.042x + 0.017$ $n = 43$
$s = 0.040$ $s = 0.0483$ $= 0.88$

While the regression coefficients differ significantly the constants do not deviate from zero) This analysis shows that the group without clinical atherosclerosis had relatively higher

) Because of the skew distribution of turbidity (y) about the lines, the regression lines of log y upon (glyceride-glycerol) were calculated and even then the regression lines differed significantly

TABLE 23. *Regression of turbidity (y) upon glyceride-glycerol (x) before (S_0) and 4 hours after (S_4) the fat meal*

Group	Sex	n	Regression equation $\begin{cases} S_0 \\ S_4 \end{cases}$	s	r	
Control	♂	26	$\hat{y} = 0.019x + 0.072$	0.00546	0.0468	0.18
			$\hat{y}_x = 0.[illegible]2x + 0.065$	0.0231	0.164	0 [illegible]
	♀	24	$\hat{y} = 0.009x + 0.064$	0.00330	0.0358	0.09
			$\hat{y} = 0.31x + 0.081$	0.0745	0.254	0.43
Hypercholesterolaemics without clinical atherosclerosis	♂	8	$\hat{y} = 0.003x + 0.133$	0.03495	0.0598	−0.03
			$\hat{y} = 0.40x - 0.12$	0.1166	0.260	0 [illegible]
	♀	22	$\hat{y} = 0.0004x + 0.089$	0.01263	0.0247	0.01
			$\hat{y} = 0.56x - 0.69$	0.0834	0.25[illegible]	0.81
Hypercholesterolaemics with clinical atherosclerosis	♂	18	$\hat{y} = 0.015x + 0.062$	0.00701	0.0527	0.46
			$\hat{y} = 0.19x + 0.12$	0.0444	0.197	0.44
	♀	10	$\hat{y} = 0.021x + 0.040$	0.00520	0.0108	0.78
			$\hat{y} = 0.12x + 0.18$	0.0620	0.187	0.51
Hyperlipaemics without clinical atherosclerosis	♂	9	$\hat{y}_x = 0.103x - 0.106$	0.00916	0.0672	0.9
			$\hat{y} = 0.56x - 1.37$	0.2042	1.036	0.68
		6	$\hat{y} = 0.020x + 0.092$	0.01412	0.0344	0.06
			$\hat{y} = 0.28x - 0.12$	0.1020	0.441	0.73
Hyperlipaemics with clinical atherosclerosis	♂	9	$\hat{y} = 0.008x + 0.138$	0.00638	0.0366	0.01
			$\hat{y} = 0.08x + 0.84$	0.0688	0.781	0.20
		6	$\hat{y}_x = 0.064x - 0.040$	0.00358	0.0[illegible]67	0.[illegible]0
			$\hat{y}_x = 0.37x - 0.47$	0.2013	0.740	0.60
Normocholesterolaemic normoglyceridaemic atherosclerotics	♂	8	$\hat{y} = 0.006x + 0.092$	0.00828	0.0090	0.61
			$\hat{y} = 0.75x - 1.57$	0.1600	0.507	0.83

fasting-serum turbidity (about twice as high) than the group with clinical atherosclerosis. This suggests that the former's chylomicron phase might have a higher glyceride-glycerol content. Hence the regression of the glyceride-glycerol level of the subnatant (y) upon that of the corresponding fasting whole serum (x) was studied in the combined groups with (1) and without (2) clinical atherosclerosis. The respective equations were

(1) $\hat{y} = 0.61x + 0.67$ $n = 46$
$s_b = 0.0472$ $s_{y.x} = 0.526$ $r = 0.89$

(2) $\hat{y} = 0.61x + 0.72$ $n = 43$
$s_b = 0.0466$ $s_y = 0.565$ $r = 0.89$

These two regression lines do not deviate significantly in either slope or level. Hence no differences was demonstrated between the groups with and without clinical atherosclerosis in either the glyceride-glycerol content of the chylomicron phase of fasting serum or

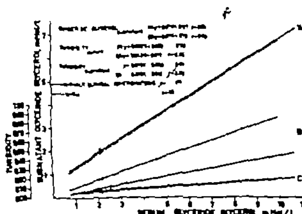


Fig. 8. Glyceride-glycerol in subnatant of fasting serum, and turbidity of serum and subnatant related to glyceride-glycerol of serum. A comparison between hypercholesterolaemics and hyperlipaemics without and with clinical atherosclerosis.

the ratio of subnatant turbidity to whole fasting-serum glyceride-glycerol level. The fact then remains that these two groups differed with respect to the turbidity of the chylomicron phase of fasting serum (cf Fig 9)

Fasting serum turbidity and glyceride-glycerol were determined in a large number of subjects either as part of general medical check-ups or in conjunction with special examinations of patients with myocardial infarction blood donors etc. These laboratory findings were supplemented with the fasting values observed in the present series of fat tolerance tests In order to estimate the screening value of serum turbidity the data were classified in order of increasing turbidity The results have been graphed in Fig 10 which is based on some 900 estimations. In each turbidity class, comprising approximately 60 estimations, the glyceride-glycerol values show considerable spread and a skew distribution. For example, in turbidity class 0 as the glyceride-glycerol values range from 0 s to 1 s mMol/l and the mean is of the order of 1 mMol/l. The corresponding figures for turbidity class 0 1s are 0 s to 3 4 and 1 s mMol/l

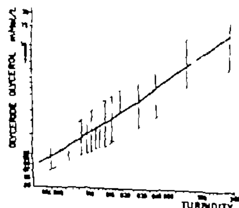


Fig. 10. Relation between glyceride-glycerol and turbidity in fasting serum. Broken lines represent 93% range

TABLE 23 *Regression of turbidity ($\hat{y}$) upon glyceride-glycerol (x) before (S_0) and 4 hours after (S_4) the fat meal*

Group	Sex		Regression equation $\hat{y}$ / $\hat{y}_t$	s	s	r
Controls	♂	36	$\hat{y} = 0.019x + 0.072$	0.00546	0.0468	0.18
			$\hat{y} = 0.22x + 0.063$	0.0231	0.164	0.74
	♀	34	$\hat{y} = 0.009x + 0.064$	0.00530	0.0358	0.00
			$\hat{y} = 0.21x + 0.031$	0.0746	0.254	0.43
Hypercholesterolaemics without clinical atherosclerosis	♂	8	$\hat{y} = 0.003x + 0.133$	0.03485	0.0598	−0.03
			$\hat{y} = 0.40x - 0.13$	0.1166	0.260	0.77
	♀	22	$\hat{y}_x = 0.0004x + 0.080$	0.01203	0.0247	0.04
			$\hat{y} = 0.56x - 0.69$	0.0854	0.257	0.81
Hypercholesterolaemics with clinical atherosclerosis	♂	18	$\hat{y}_x = 0.015x + 0.062$	0.00301	0.0527	0.48
			$\hat{y} = 0.19x + 0.13$	0.0444	0.197	0.44
	♀	10	$\hat{y} = 0.021x + 0.049$	0.00520	0.0103	0.78
			$\hat{y}_x = 0.12x + 0.18$	0.0620	0.187	0.51
Hyperlipaemics without clinical atherosclerosis	♂	9	$\hat{y}_x = 0.103x - 0.106$	0.00916	0.0672	0.9
			$\hat{y} = 0.56x - 1.37$	0.2042	1.056	0.68
	♀	6	$\hat{y} = 0.020x + 0.093$	0.01412	0.0344	0.06
			$\hat{y} = 0.28x - 0.13$	0.1020	0.441	0.73
Hyperlipaemics with clinical atherosclerosis	♂	9	$\hat{y} = 0.008x + 0.128$	0.00638	0.0366	0.04
			$\hat{y} = 0.08x + 0.84$	0.0888	0.781	0.29
	♀	6	$\hat{y} = 0.064x - 0.040$	0.00354	0.0267	0.90
			$\hat{y} = 0.37x - 0.47$	0.2013	0.740	0.60
Normocholesterolaemic, normoglyceridaemic atherosclerotic	♂	8	$\hat{y} = 0.006x + 0.092$	0.00828	0.0090	0.61
			$\hat{y} = 0.75x - 1.37$	0.1600	0.507	[illegible]

fasting-serum turbidity (about twice as high) than the group with clinical atherosclerosis This suggests that the former a chylomicron phase might have a higher glyceride-glycerol content Hence the regression of the glyceride glycerol level of the subnatant (y) upon that of the corresponding fasting whole serum (x) was studied in the combined groups with (1) and without (2) clinical atherosclerosis The respective equations were

(1) $\hat{y} = 0.61x + 0.67$ $n = 45$
$s_b = 0.0473$ $s_y = 0.526$ $r = 0.89$

(2) $\hat{y} = 0.61x + 0.72$ $n = 43$
$s_b = 0.0466$ $s_y = 0.565$ $r = 0.89$

These two regression lines do not deviate significantly in either slope or level Hence no differences was demonstrated between the groups with and without clinical atherosclerosis in either the glyceride-glycerol content of the chylomicron phase of fasting serum or

TABLE ** *Regression of serum glyceride-glycerol (y) upon subnatant glyceride-glycerol (x) 4 hours after the fat meal*

Group	Sex		Regression equation			
Controls	♂	38	$\hat{y} = 0.6_x + 0.39$	0.036	0.[illegible]40	0.84
		31	$\hat{y} = 0.84x - 0.01$	0.044	0.150	0.95
Hypercholesterolaemics without clinical atherosclerosis	♂	8	$\hat{y} = 0.70x + 0.18$	0.0[illegible]	0.120	0.94
		12	$\hat{y} = 0.80x + 0.25$	0.030	0.178	0.93
Hypercholesterolaemics with clinical atherosclerosis	♂	18	$\hat{y} = 0.61x + 0.77$	0.030	0.250	0.90
		10	$\hat{y} = 0.78x - 0.27$	0.044	0.[illegible]34	0.93
Hyperlipaemics without clinical atherosclerosis	♂	9	$\hat{y} = 0.31 \; + 3.02$	0.108	0.342	0.70
		6	$\hat{y} = 0.89x + 0.03$	0.134	0.540	0.94
Hyperlipaemics with clinical atherosclerosis	♂	9	$\hat{y} = 0.33x + 1. \; 3$	0.0 0	0.617	0.81
		6	$\hat{y} = 0.75x - 0.00$	0.1[illegible]6	0.573	0.9[illegible]
Normocholesterolaemic normoglyceridaemic atherosclerotics	♂	8	$\hat{y} = 0. \; 4x - 0.24$	0.117	0.270	0.81

0.28 mMol/l at whole serum glyceride-glycerol level of 02 and 1.78 mMol/l. In the latter the corresponding values were 1.28 and 0. 6 mMol/l and 4.08 and 4.18 mMol/l respectively.

The regression of the glyceride-glycerol of the subnatant upon that of whole 4-hour serum for males and females in each group will be found in Table **. The line of regression for men differs significantly in slope from that for women in each group except among hypercholesterolaemics without clinical atherosclerosis. But no difference appeared between the combined groups with and without clinical atherosclerosis. When the entire series was classified by sex only the regressions of the glyceride-glycerol of the subnatant upon that of whole serum were as shown in Fig. 11. Among females there was no significant difference between the controls, hypercholesterolaemics and hyperlipaemics, so in Fig. 6 these are represented by a single line having the equation

$$\hat{y} = 0.77x + 0.13 \quad n = 8$$
$$s = 0.024 \quad s_{_} = 0.328 \quad r = 0.96$$

For the male groups the lines representing the regression of the glyceride-glycerol level of the subnatant upon that of whole serum disclosed that the line for the hypercholesterolaemics deviated significantly in level and that for the hyperlipaemics in slope from the other lines (cf. Fig. 11). Accordingly neither classification by the glyceride-glycerol of whole 4-hour serum nor analysis of the regression of the glyceride-glycerol of the subnatant upon that of whole 4-hour serum disclosed any significant difference between the groups with and without clinical atherosclerosis. But there was a significant difference between men and women.

Conversion of the glyceride-glycerol values into logarithms yields a more normal distribution within turbidity classes The means and ranges shown in the curve were calculated from such logarithms The curve was constructed after twice calculating successive means and ranges and reconverting these into the corresponding glyceride-glycerol values As appears from Fig 10 the turbidity and glyceride-glycerol level of fasting serum are to some extent associated but the great spread severely limits the screening value of the turbidity measurements. At serum turbidity values not exceeding 0 08 none of the 140 glyceride-glycerol values was over 2 mMol/l and as many as 50 per cent were below 1 mMol/l The chance that a single turbidity measurement of 0 14 will be accompanied by a glyceride glycerol level exceeding 2 mMol/l is about 50 per cent and it will not exceed 5 mMol/l

Glyceride-glycerol of whole serum and its subnatant 4 hours after the fat meal

Mean glyceride-glycerol of whole serum and its subnatant as well as their difference — the chylomicron phase — 4 hours after the fat meal are specified for each group in Table 7 page 23 As appears from the table the mean glyceride-glycerol of whole 4 hour serum for males exceeded that for females in each group Moreover the glyceride-glycerol of the chylomicron phase was higher when the glyceride glycerol of whole serum was high than when it was low To compare the glyceride-glycerol of the chylomicron phase in males and females the series was classified according to the glyceride-glycerol of whole serum and the mean glyceride-glycerol of the subnatant in per cent of the mean glyceride-glycerol of whole serum was calculated for males and females in each such class up to whole 4 hour serum glyceride-glycerol of 6 mMol/l See Table 26 The subnatant was relatively higher for women than for men in each group In other words the males had a larger chylomicron phase than the females

In the combined groups with whole serum glyceride-glycerol of 0—3 and of 3—6 mMol/l the glyceride-glycerol of the chylomicron phases for men and women differed significantly (χ^2 >3 84) In the first group the mean chylomicron phases were 0 44 and

TABLE 26 *Glyceride-glycerol in subnatant in per cent of glyceride-glycerol serum 4 h after (%) the fat meal*

Sex	S-classes, mMol/l					
	0–1	1–2	2–3	3–4	4–	6
Men	86	79	7	[illegible]	69	[illegible]
n =	3	19	20	1	7	3
Women	87	82	84	[illegible]	83	[illegible]
n =	3	24	23	7	1	5

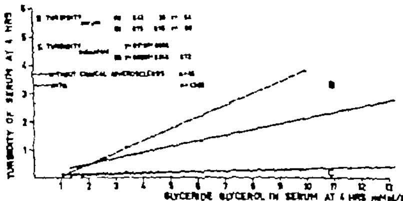


Fig. 12. Glyceride-glycerol in subnatant of serum 4 hour after the fat meal, and turbidity of serum and subnatant, related to glyceride-glycerol of serum. A comparison between hypercholesterolaemics and hyperlipaemics without and with clinical atherosclerosis.

significant regression of turbidity upon glyceride-glycerol of whole serum existed. In this respect no difference could be demonstrated between men and women. Groups with clinical atherosclerosis differed from those without, however, in as much as the turbidity relative to the glyceride-glycerol level was lower in the former than in the latter. The importance of this definite difference may be somewhat questioned by the different average age between groups with and without clinical atherosclerosis.

Glyceride-glycerol of whole serum and its subnatant 7 hours after the fat meal

As appears from Table 7, page 23, the glyceride-glycerol of whole 7-hour serum and of its subnatant differed significantly for the male controls, female controls, hypercholesterolaemics and hyperlipaemics. Since analysis demonstrated no differences between men and women or between groups with and without clinical atherosclerosis with respect to the regression of the glyceride-glycerol of the subnatant upon that of whole 7-hour serum, this relationship was studied in (1) the controls regardless of sex, (2) a group consisting of all hypercholesterolaemics and normocholesterolaemic normoglyceridaemic atherosclerotics and (3) a group comprising all hyperlipaemics. The lines for the regression of the glyceride-glycerol level of the subnatant (y) upon that of whole 7 hour serum (x) in these groups are shown in Fig. 12 and have the following equations:

(1) $y = 0.8x + 0.002$; $n = 41$
$s = 0.046$ $s_{yx} = 0.149$ $r = 0.92$

(2) $y = 0.9x + 0.002$; $n = 1$
$s = 0.034$ $s_{yx} = 0.203$ $r = 0.98$

(3) $y = 0.74x + 0.27$ $n = 10$
$s = 0.128$ $s = 0.473$ $r = 0.88$

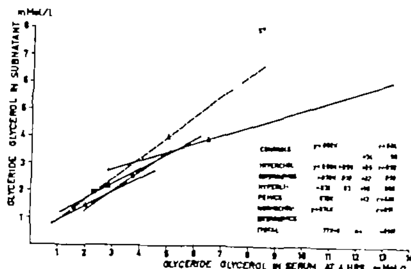


Fig 11 Rel tion between glyceride-glycerol in serum 4 hours fter the fat meal and it subnat t (25 000 g) in men (———) nd w men ( )

Turbidity and glyceride-glycerol of whole 4-hour serum

The regressions of the turbidity upon the glyceride-glycerol of whole 4 hour serum for males and for females in the aforementioned groups regardless of the presence of clinical atherosclerosis *showed no statistically significant differences* Therefore men and women were combined and the regression of turbidity (y) upon the glyceride-glycerol of whole 4 hour serum became

$$\hat{y} = 0\ 30x - 0\ 09 \quad n = 100$$
$$s_b = 0\ 021 \quad s_y = 0\ 311 \quad r = 0\ \ 4$$

The constant in this equation differs significantly from zero

However the regression of turbidity upon glyceride glycerol of whole 4 hour serum showed a significant difference between those with and those without clinical atherosclerosis among the hypercholesterolaemics as well as among the hyperlipaemics The regressions of the turbidity (y) upon the glyceride-glycerol (x) of whole 4 hour serum in the combined hypercholesterolaemic and hyperlipaemic groups without (1) and with (2) clinical atherosclerosis (Fig 12) have the equations

$$(1) \quad \hat{y} = 0\ 42x - 0\ 39 \quad n = 45$$
$$s_b = 0\ 040 \quad s_y = 0\ 581 \quad r = 0\ 84$$

$$(2) \quad \hat{y} = 0\ 10x + 0\ 15 \quad n = 47$$
$$s_b = 0\ 040 \quad s_y = 0\ 58 \quad r = 0\ 60$$

The slopes of these regression lines deviate significantly [1])

This differences between the groups with and without clinical atherosclerosis has no corollary in either the subnatant or the chylomicron phase of 4 hour serum

In sum then the following can be said about the association between the turbidity and glyceride glycerol of serum 4 hours after the fat meal A

[1]) See footnot p 37

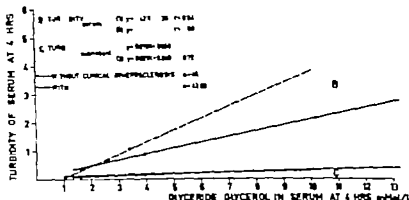


Fig. 12. Glyceride-glycerol in subnatant of serum 4 hours after the fat meal, and turbidity of serum and subnatant, related to glyceride-glycerol of serum. A comparison between hypercholesterolaemics and hyperlipaemics without and with clinical atherosclerosis.

significant regression of turbidity upon glyceride-glycerol of whole serum existed. In this respect no difference could be demonstrated between men and women. Groups with clinical atherosclerosis differed from those without however in as much as the turbidity relative to the glyceride-glycerol level was lower in the former than in the latter. The importance of this definite difference may be somewhat questioned by the different average age between groups with and without clinical atherosclerosis.

Glyceride-glycerol of whole serum and its subnatant 7 hours after the fat meal

As appears from Table 7 page 23 the glyceride glycerol of whole 7-hour serum and of its subnatant differed significantly for the male controls, female controls, hypercholesterolaemics and hyperlipaemics. Since analyses demonstrated no differences between men and women or between groups with and without clinical atherosclerosis with respect to the regression of the glyceride-glycerol of the subnatant upon that of whole 7 hour serum this relationship was studied in (1) the controls regardless of sex, (2) a group consisting of all hypercholesterolaemics and normocholesterolaemic normoglyceridaemic atherosclerotics and (3) a group comprising all hyperlipaemics. The lines for the regression of the glyceride-glycerol level of the subnatant (y) upon that of whole 7 hour serum (x) in these groups are shown in Fig. 13 and have the following equations:

(1) $\hat{y} = 0.87x + 0.003$ $n = 1$
$s_b = 0.048$ $s_{y.x} = 0.140$ $r = 0.92$

(2) $\hat{y} = 0.98x + 0.002$ $n = 1$
$s_b = 0.034$ $s_{y.x} = 0.\ 05$ $r = 0.98$

(3) $\hat{y} = 0.75x + 0.27$ $n = 10$
$s = 0.128$ $s = 0.473$ $r = 0.88$

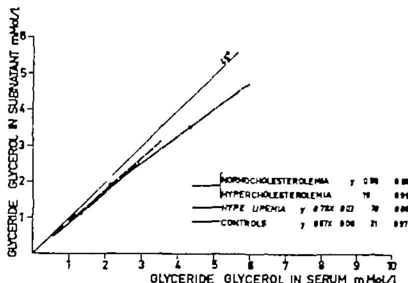


Fig 13 Relation between glyceride glycerol in serum and it subnata t (25 000 g) hours after the f t meal.

The constant does not differ from zero in any of these three equations nor are their regression coefficients significantly unequal and only that for the controls deviates significantly from unity

When the subjects were classified according to whole 7 hour serum glyceride-glycerol levels of 0—2 ($n = 32$) 2—4 ($n = 13$) and 4—6 ($n = 7$) mMol/l the mean subnatant glyceride glycerol levels in per cent of the corresponding whole serum means were found to be 89 87 and 81 per cent respectively

Turbidity and glyceride-glycerol of whole 7 hour serum

The regression of turbidity (y) upon glyceride glycerol level (x) of whole 7 hour serum for (1) controls (2) hypercholesterolaemics and hyperlipaemics without clinical atherosclerosis and (3) all subjects with clinical atherosclerosis has the following equations

(1) $y = 0\ 10x + 0\ 08$ $n = 21$
$s_b = 0\ 026$ $s_y = 0\ 084$ $r = 0\ 6$

(2) $y = 0\ 12x + 0\ 13$ $n = 12$
$s_b = 0\ 04$ $s_{y.x} = 0\ 70$ $r = 0\ 60$

(3) $y = 0\ 12x + 0\ 05$ $n = 19$
$s_b = 0\ 00$ $s_y = 0\ 234$ $r = 0\ 56$

These regression lines do not differ significantly in either slope or level

The regressions of turbidity upon glyceride-glycerol level of serum immediately before as well as 4 and 7 hours after the fat meal are illustrated in Fig 14 The slopes of these regression lines differ significantly But the corresponding subnatants exhibited no difference in this respect Hence the difference was to be found in the chylomicron phase where the following regressions of turbidity (y) upon glyceride-glycerol (x) were obtained for

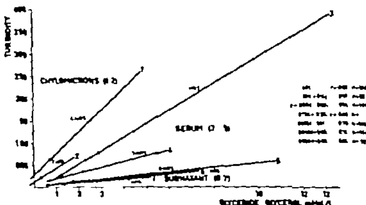


Fig 14. Relation between turbidity and glyceride-glycerol in serum, in its supernatant and chylomicron phase before 4 and 7 hours after the fat meal.

the chylomicron phase of (1) 4-hour serum and ( ) 7 hour serum

(1) $y = 0.48x + 0.27$, $n = 166$
$s_b = 0.044$, $s_{yx} = 0.646$, $r = 0.66$

(2) $y = 0.30x + 0.14$, $n = 62$,
$s_b = 0.078$, $s_{yx} = 0.219$, $r = 0.47$

Both the slopes and the levels of these lines deviate significantly). Accordingly at equal glyceride-glycerol levels of the chylomicron phase 4 hour serum seems to have greater chylomicron turbidity than 7-hour serum in these groups.

) See footnote p. 57

CHAPTER IX

EFFECT OF DIETETIC CHANGES ON THE FAT TOLERANCE TEST

Definition of diets

Ordinary diet in the present context means the patient s ordinary diet or the regular hospital diet both of which contain predominantly fats of the saturated type

Low-saturated fats diet implies that the patient was asked to refrain from eating ordinary milk cream butter cheese eggs fat meats etc Most subjects on this diet lost a few kilos in body weight suggesting that it provided a somewhat lowered caloric intake

High poly unsaturated fats diet The low saturated fats diet with a daily supplement of 50 ml of oil with a minimum poly unsaturated fats content of 55 per cent

Technique for repeated fat tolerance tests

The fat tolerance test on ordinary diet was a rule made while the patient was admitted to hospital for a general check up Upon discharge the patient was put on a low-saturated fats diet for six weeks whereupon the fat tolerance test was repeated in the out patient department Then the patient was put on a high poly unsaturated fats diet for another six weeks and the fat tolerance test was repeated once more in the out patient department.

Fat tolerance tests on ordinary diet and on a high poly unsaturated fats diet

The alterations in the fat tolerance test resulting after a change from ordinary diet to a high poly unsaturated fats diet was studied in 32 subjects 16 hypercholesterolaemics and 16 hyperlipaemics. Mean cholesterol phospholipid glyceride-glycerol and turbidity values for fasting and 4 hour sera at the repeated fat tolerance tests are presented in Table 28 The table reveals that the means of all lipid factors in serum immediately before the fat meal were significantly reduced when the patients fat intake was changed from saturated to poly unsaturated fats

The regression of the serum glyceride-glycerol immediately before (x) on that 4 hours after (y) the fat meal when the same patient s fat tolerance test on ordinary diet (1) was repeated on a high poly unsaturated fats diet (2) has the equations

(1) $y = 1\ 30x + 0\ 67 \quad n = 32$
$s_b = 0\ 069 \quad s_{y,x} = 1\ 05 \quad r = 0\ 93$

(2) $y = 1\ 28x + 0\ 44 \quad n = 32$
$s_b = 0\ 080 \quad s_{y,x} = 0\ 56 \quad r = 0\ 92$

Neither the coefficients of regression nor the constants were significantly unequal The differences between the means of x (3 06 and 1 86 mMol/l re

TABLE 21. *Fat tolerance test on 21 subjects on ordinary diet and repeated on diet restricted in saturated fat and poly-unsaturated fat added*

	Cholesterol mg per cent		Phospholipids mg per cent		Glyceride glycerol m Mol/l		Turbidity	
	before	4 hrs	before	4 hrs	before	4 hrs	before	4 hrs
Ordinary diet	360 1	364 0	333.1	360.5	3 05	4 64	0 16	1 18
Diet with poly unsaturated fat	310 2	308 0	300.5	313 2	1.86	2 83	0 11	0 63
Difference ($\bar{d}$)	50.9	56 0	32.6	45.	1 19	1.81	0 05	0.5
	12.6	9 7	11 1	10.6	0.39	0 44	0 02	0 19
	4 03	5.79	2 91	4.27	4 09	4 14	2.50	2 96
Decrease in per cent	14	15	10	12	39	39	33	4

pectively) and the means of y (4 64 and 2 83 mMol/l respectively) are significant

Unlike the case of duplicate fat tolerance tests (cf Table 14 p 35) the fasting serum exhibited no significant association between either cholesterol or phospholipids or turbidity on the one hand and glyceride-glycerol on the other when the fat tolerance test was repeated after substitution of saturated fats by poly unsaturated fats (χ^2 test) This phenomenon might conceivably be accounted for by the possibility that such a change in the patient s diet occasionally is attended by dissociation of the cholesterol phospholipid and glyceride-glycerol changes. For example at the repeated fat tolerance tests a man of 51 had fasting sera with cholesterol levels of 307 and 293 mg %, phospholipid levels of 323 and 301 mg % and glyceride-glycerol levels of 8.58 and 3 08 mMol/l. Similarly on the two occasions the fasting serum of a woman of 53 showed cholesterol values of 441 and 308 mg %, phospholipid values of 422 and 307 mg %, glyceride-glycerol values of 2.58 and 28 mMol/l and turbidity values of 0 13 and 0 07

In 15 cases (8 hypercholesterolaemics and 7 hyperlipaemics) similar comparisons were made between the fasting 4 hour and 7 hour values As appears from Table 20 the greatest differences between fat tolerance tests repeated on different diets appeared 7 hours after the fat meal while the fasting values exhibited the smallest differences. Whereas the turbidity values in either fasting or 4-hour or hour sera showed no significant changes, significant decreases were observed in all 4 hour and 7 hour values for cholesterol, phospholipids and glyceride-glycerol.

The regression of the glyceride-glycerol level of 7-hour serum (y) upon that of fasting serum (x) when the fat tolerance test was repeated after a change from ordinary diet (1) to a

CHAPTER IX

EFFECT OF DIETETIC CHANGES ON THE FAT TOLERANCE TEST

Definition of diets

Ordinary diet in the present context means the patient's ordinary diet or the regular hospital diet both of which contain predominantly fats of the saturated type

Low-saturated fats diet implies that the patient was asked to refrain from eating ordinary milk, cream butter cheese, eggs fat meats, etc Most subjects on this diet lost a few kilos in body weight suggesting that it provided a somewhat lowered caloric intake.

High poly-unsaturated fats diet The low saturated fats diet with a daily supplement of 50 ml of oil with a minimum poly unsaturated fats content of 55 per cent

Technique for repeated fat tolerance tests

The fat tolerance test on ordinary diet was a rule made while the patient was admitted to hospital for a general check up Upon discharge the patient *was put on a low-saturated fats diet* for six weeks whereupon the fat tolerance test was repeated in the out patient department Then the patient was put on a high poly unsaturated fats diet for another six weeks and the fat tolerance test was repeated once more in the out patient department

Fat tolerance tests on ordinary diet and on a high poly unsaturated fats diet

The alterations in the fat tolerance *test resulting after a change from* ordinary diet to a high poly unsaturated fats diet was studied in 32 subjects 16 hypercholesterolaemics and 16 hyperlipaemics. Mean cholesterol phospholipid glyceride-glycerol and turbidity values for fasting and 4-hour sera at the repeated fat tolerance tests are presented in Table 28 The table reveals that the means of all lipid factors in serum immediately before the fat meal were significantly reduced when the patients fat intake was changed from saturated to poly unsaturated fats.

The regression of the serum glyceride-glycerol immediately before (x) on that 4 hours after (y) the fat meal when the same patient's fat tolerance test on ordinary diet (1) was repeated on a high poly unsaturated fats diet (2) has the equations

(1) $y = 1\ 80x + 0\ 67 \quad n = 32$
$s_b = 0\ 060 \quad s_{y.x} = 1\ 05 \quad r = 0\ 93$

(2) $y = 1\ 28x + 0\ 44 \quad n = 32$
$s_b = 0\ 080 \quad s_{y.x} = 0\ 56 \quad r = 0\ 91$

Neither the coefficients of regression nor the constants were significantly unequal The differences between the means of x (3 06 and 1 86 mMol/l re

TABLE 30. *Cholesterol and phospholipid changes in subjects showing decrease in fasting serum of at least 20 mg% on diet restricted in saturated fat and poly-unsaturated fat added*

	Cholesterol mg%			Phospholipid mg%		
	N	PUFA	N−PUFA	N	PUFA	N−PUFA
$\bar{x}$	290 ± 1	292	+88	337 ± 19	[illegible]	− 1
A: Diff $\bar{x}$	− 1 1	+ 3.5	− 4 6	± 6.3	+ 18 4	− 8.1
s diff	31 0	7.3	21 1	33 3	9 5	29.3
diff sign ≠ 0	no	yes	no	yes	yes	no
$\bar{x}$	298 ± 11	296	9	367 ± 9	290	6
B Diff $\bar{x}$	6 0	− 2 9	− 3.1	+ 21	+ 12 6	− 19 1
σ	30 8	22.9	45.1	32 4	1 4	40 4
diff. sign ≠ 0	no	no	no	[illegible]	no	no

N = Ordinary diet with saturated fat

PUFA = Diet restricted in saturated fat and poly-unsaturated fat added

difference in the spread of both the cholesterol and the phospholipid changes from immediately before to 4 hours after the fat meal. In both cases this spread was significantly narrower on the diet rich in poly unsaturated fats. This could be a manifestation of more stable metabolic conditions on the latter diet.

Corresponding studies for fasting and 7 hours values were made in a cholesterol group of 7 hypercholesterolaemics and 4 hyperlipaemics and a phospholipid group of 5 hypercholesterolaemics and 4 hyperlipaemics. The results are presented in Table 30 b. It discloses no differences between the serum cholesterol changes. Phospholipids were significantly higher in hour serum than in fasting serum on ordinary diet but not on a high-poly unsaturated fats diet. No significant difference in the phospholipid change could be demonstrated. The spread of the cholesterol and phospholipid changes were smaller on the latter diet (significantly so for cholesterol only).

Fat tolerance tests on ordinary diet and on a low-saturated fats diet

Fat tolerance tests repeated after a change from ordinary diet to a low saturated-fats diet were compared in 13 cases, 5 hypercholesterolaemics and 8 hyperlipaemics. Both immediately before and 4 hours after the fat meal, the serum cholesterol phospholipid, glyceride-glycerol and turbidity means

TABLE 29 *Serum l pids before 4 and 7 hours after th fat meal in 8 hypercholesterolaemic nd hyperl paemics on ord nary diet (N) nd on d et restricted i sat rated fat and poly-un t rated fat added (PUFA)*

	Cholesterol mg%				Phospholipid mg %				Glyceride-glycerol mMol/l				Turbidity			
	Diet N	Diet PUFA	diff	t_{diff}	Diet N	Diet PUFA	diff	t_{diff}	Diet N	Diet PUFA	diff	t_{diff}	Diet N	Diet PUFA	diff	t_{diff}
0 hr	383	3 4	39.2 ±0.2	93	341	314	26. 16.4	1 62	.50	1 75	0.75 0.30	.53	0 14	0.17	−0 0?0 0 018	1 [illegible]
4 hrs	387	322	65 7 12 9	5.16	371	328	42.3 14.3	3.03	3 80	.73	1 07 0 46	2.33	0.88	0.[illegible]	0 113 0 ?01	0.5
7 hrs	385	316	70 9 11 8	6 01	390	320	51 0 13 3	3.83	3 56	2 04	1.52 0 [illegible]	5 30	0 93	0 47	0 462 0 234	1 8[illegible]

± standard deviation

high polyunsaturated fats diet (2) satisfied the equations

(1) $y = 1\ 21x + 0\ 54 \quad n = 15$
$s_b = 0\ \ 1 \quad s_y = 1\ 336 \quad r = 0\ 75$

(2) $y = 1\ \ 6x + 0\ 8 \quad n = 15$
$s_b = 0\ 267 \quad s_{y.x} = 0\ \ 8 \quad r = 0\ 77$

The lines corresponding to these equations did not differ significantly in either slope or level Corresponding mean serum glyceride-glycerol level before and 7 hours after the fat meal differed significantly Similar regressions of the serum glyceride-glycerol level 7 hours after (x) upon that 4 hours after (y) when the fat intake was changed from saturated (1) to poly unsaturated fats (2) were compared The following equations were obtained

(1) $y = 1\ 01x - 0\ 28 \quad n = 15$
$s_b = 0\ 1\ \ 8 \quad s_y = 0\ 697 \quad r = 0\ 90$

(2) $y = 0\ 84x + 0\ 05 \quad n = 15$
$s_b = 0\ 0 \quad s_{y.x} = 0\ 388 \quad r = 0\ 95$

Nor did these lines deviate in either slope or level

As a corollary to what was done with the duplicate fat tolerance tests (Chapter V page 32) those cases were studied in which the dietic change caused the repeated fat tolerance test to show a *fasting serum cholesterol* decrease ($n = 21$) and/or phospholipid decrease ($n = 10$) exceeding 20 mg The cholesterol group comprised 1 hypercholesterolaemics and 0 hyperlipaemics and the phospholipid group 0 hypercholesterolaemics and 10 hyperlipaemics. The results are presented in Table 30 a The table reveals that such significant differences in the cholesterol and phospholipid changes from immediately before to 4 hours after the fat meal as could be demonstrated at the duplicate fat tolerance test did not appear when the fat tolerance test was repeated on a high poly unsaturated fats diet On the other hand there emerged a significant

TABLE 31 *Fat tolerance test on 15 subjects on ordinary diet and on diet restricted in saturated fa*

	Cholesterol mg%		Phospholipid mg%		Glyceride-gly mMol l		Turbid y	
	before	4 hrs	before	4 hrs	before	4 hrs	before	4 hrs
Ordinary diet	315	216	300	3[illegible]	3 03	4.82	0 19	1 41
Restricted diet	294	262	294	313	2 62	2.75	0 11	0.70
Difference ($\bar{d}$)	21 7	15 8	11 9	14.2	0 63	1.38	0.070	0.742
	15.8	16 2	11 7	1 .2	0 41	0 81	0.041	0.[illegible]45
t-test of $\bar{d}$	1.37	0 97	0 91	0.83	1.50	1.25	1 [illegible]	3 03
Decrease in %	7	6	4	4	17	7	39	3

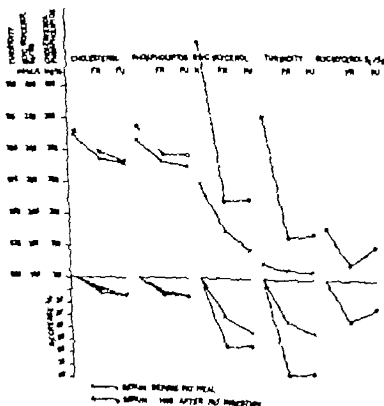


Fig 15. Standard fat meal on ordinary diet (O), diet restricted in saturated fat (FR) nd FR diet w h poly-unsaturated fat added (PU).

were lower after restriction of saturated fats but the turbidity of 4 hour serum exhibited the only significant difference (Table 31)

The regression of the same subject s serum glyceride-glycerol level 4 hours after the fat meal (y) upon that immediately before it (x) fitted the following equations on ordinary diet (1) and on a low saturated fats diet (2)

$$(1) \quad \bar{y} = 0\ 05x+2\ 13 \quad n = 13$$
$$s_b = 0\ 105 \quad s_{y,x} = 1\ 380 \quad r = 0\ 80$$

$$(2) \quad \bar{y}_x = 1\ 30x+0\ 4 \quad n = 13$$
$$s_b = 0\ \ 94 \quad s_y \ = 1\ 368 \quad r = 0\ 78$$

These regression lines did not deviate significantly in either slope or level Hence the association between the serum glyceride-glycerol level 4 hours after and immediately before the fat meal on ordinary diet seemed substantially similar to that on a low saturated fats diet The regression line corresponding to equation (2) is a good approximation of the corresponding line for the larger series ($n = 32$) on ordinary diet

In only 6 subjects (3 hypercholesterolaemics and 3 hyperlipaemics) was the fat tolerance test on ordinary diet repeated on a low-saturated fats diet then once more on a high poly unsaturated fats diet Means for lipids and turbidity immediately before and 4 hours after the fat meal on the three diets are shown in Fig 15 revealing that the greatest serum lipid reduction occurred on the diet with restricted saturated fats while the subsequent addition of poly unsaturated fats diet maintained the levels or reduced them slightly more Cholesterol and phospholipids exhibited the lowest percentage decreases (10—15%) while the glyceride-glycerol and turbidity of serum were reduced 30—40 per cent immediately before and a bit more 4 hours after the fat meal

Summarizing the following can be stated

A When fat tolerance tests on ordinary diet were repeated on a high poly unsaturated fats diet and the fasting serum lipids and turbidity decreased significantly the mean differences in lipids and turbidity between the 7 hour sera were larger and had smaller standard deviations than those for the fasting sera This suggests that in this respect the fat tolerance test could be of some use

B The cholesterol and phospholipid changes from immediately before to 4 hours after the fat meal were greater on ordinary diet than on a diet rich in poly unsaturated fats (not significant) and their spread is significantly smaller in the latter case

C The largest reductions in serum lipids and turbidity are induced by a diet with restriction of saturated fats and addition to that diet of supplementary poly unsaturated fats maintains or slightly accentuates these reductions.

Comment

The results of a fat tolerance test are highly dependent on the subject s diet during the period preceeding the test Whereas isocaloric fat restriction increased the fat tolerance (POME

CHAPTER X

GENERAL DISCUSSION

For the last 50 years fat tolerance tests involving lipid estimations, chylomicron counts and turbidity measurements in serum have been performed in a variety of ways. Balanced test meals combining both carbohydrates and proteins with fat have been used very widely. Such meals would no doubt be preferable were it not for the fact that the serum triglycerides are influenced not only by carbohydrates but also in all probability by proteins. And the introduction of so many imponderables must make it exceedingly difficult to draw valid conclusions. A standard meal composed exclusively of fats would seem to be superior in yielding reproducible experiments.

Cold double cream proved to be a well tolerated and readily standardizable meal. It might seem a good idea to adjust the amount of fat administered to the weight of the subject, but the present investigation did not demonstrate any association between the serum lipids neither immediately before nor 4 hours after a standard fat meal and body weight, stature or body surface area.

Reproducibility. The majority of previous workers have expressed the view that fat tolerance tests yield reasonably reproducible results. The methods of choice has been to plot curves delineating the individual's lipid changes in serum at duplicate fat tolerance tests and these have been accepted as similar. On the other hand, no previous author seems to have attempted to estimate the magnitude of the variations. The present investigation revealed that the serum lipids and the turbidity before and 4 hours after the fat meal are associated. In analysis of the changes in serum induced by a fat meal, the first step was to estimate the range of normal variation of the serum lipids and turbidity before the fat meal in three groups of patients: hypercholesterolaemics, hyperlipaemics and normocholesterolaemic normoglyceridaemics. The standard deviation in per cent of the mean was 10—11 per cent for cholesterol, 1—20 per cent for phospholipids, 21—73 per cent for glyceride-glycerol, and 64—235 per cent for turbidity.

Considering that the lipids and turbidity vary widely even in fasting serum, one might well ponder whether there is any point in talking about such a thing as reproducibility of a fat tolerance test. — When subjects with a large glyceride-glycerol difference in fasting serum were selected, it turned out that the ratio of the serum glyceride-glycerol level 4 hours after

BANZE BRINFIELD & CHESSIN 1954 HAVEL 1957 BRONTE-STEWART & BLACKBURN 1958) an increase of the dietary fat intake from 40 to 54 per cent for 3 weeks did not affect the fat tolerance (HORLICK, 1957)

Administration of poly unsaturated fats to subjects with ischaemic heart disease and high turbidity curves caused their fat tolerance to become more like that of the controls (BRONTE-STEWART & BLACKBURN 1958)

The writer's observations bear out previous findings In the present investigation isocaloric fat restriction was not attempted but merely restriction of saturated fats This means that the relation between saturated and unsaturated fats was shifted in favour *of unsaturated dietary fats Hence the* caloric intake was reduced as manifested by the fact that the patients lost some 2 kg body weight over the *period of 6 weeks they were on the* low-saturated fats diet. When they subsequently received supplementary poly unsaturated fats in the diet their body weight generally returned to what it had been on ordinary diet The increased fat tolerance persisted or was accentuated after addition to the low-saturated fats diet of supplementary poly unsaturated fats As a measure of the fat tolerance the lipid values of 7 hour seemed superior

The analysis of duplicate fat tolerance tests (cf Chapter 5) disclosed that the test with the lower fasting value showed the greater cholesterol or phospholipid change 4 hours after the fat meal Conversely when the fat tolerance test on ordinary diet was repeated *on a high poly unsaturated fats diet* the test with the lower fasting-serum phospholipid level showed if anything the smaller phospholipid change

CHAPTER X

GENERAL DISCUSSION

For the last 50 years fat tolerance tests involving lipid estimations chylomicron counts and turbidity measurements in serum have been performed in a variety of ways. Balanced test meals, combining both carbohydrates and proteins with fat have been used very widely. Such meals would no doubt be preferable were it not for the fact that the serum triglycerides are influenced not only by carbohydrates but also in all probability by proteins. And the introduction of so many imponderables must make it exceedingly difficult to draw valid conclusions. A standard meal composed exclusively of fats would seem to be superior in yielding reproducible experiments.

Cold double cream proved to be a well tolerated and readily standardizable meal. It might seem a good idea to adjust the amount of fat administered to the weight of the subject, but the present investigation did not demonstrate any association between the serum lipids neither immediately before nor 4 hours after a standard fat meal and body weight stature or body surface area.

Reproducibility. The majority of previous workers have expressed the view that fat tolerance tests yield reasonably reproducible results. The methods of choice has been to plot curves delineating the individual's lipid changes in serum at duplicate fat tolerance tests and these have been accepted as similar. On the other hand, no previous author seems to have attempted to estimate the magnitude of the variations. The present investigation revealed that the serum lipids and the turbidity before and 4 hours after the fat meal are associated. In analysis of the changes in serum induced by a fat meal the first step was to estimate the range of normal variation of the serum lipids and turbidity before the fat meal in three groups of patients: hypercholesterolaemics, hyperlipaemics and normocholesterolaemic normoglyceridaemics. The standard deviation in per cent of the mean was 10—11 per cent for cholesterol, 12—20 per cent for phospholipids 21—72 per cent for glyceride-glycerol, and 64—230 per cent for turbidity.

Considering that the lipids and turbidity vary widely even in fasting serum one might well ponder whether there is any point in talking about such a thing as reproducibility of a fat tolerance test. — When subjects with a large glyceride-glycerol difference in fasting serum were selected, it turned out that the ratio of the serum glyceride-glycerol level 4 hours after

the fat meal to that immediately before it remained substantially constant from one occasion to another In other words the relative glyceride glycerol rise on different occasions was of similar magnitude If this fact is utilized for estimation of the "standard error" of a single fat tolerance test it is found that this is of the order of 20 per cent And a "standard error" of this order requires that the groups of patients under study differ a good deal or that the groups are very large

The individual's response to the fat meal An individual's response to the fat meal can be studied by letting him undergo duplicate fat tolerance tests With the aid of this method the present investigation disclosed that for one and the same individual on the same diet on both occasions the turbidity readings can serve as a screening test — the higher turbidity is usually accompanied by the higher glyceride glycerol level Furthermore the higher turbidity value and the higher glyceride glycerol value of fasting serum are as a rule both accompanied by the corresponding higher value for serum 4 hours after the fat meal It was demonstrated with respect to serum cholesterol as well as to serum phospholipids that the lower of the two fasting values undergoes the greatest change in response to the fat meal The serum cholesterol decreases occasionally observed in response to the fat meal could be responsible for the absence of appreciable cholesterol changes in the majority of investigations — the present one included — concerned with groups of patients

Fat tolerance tests in different categories of subjects The controls constituted a highly important group in the present investigation in so far as it was the sole group with random lipid factors in serum All the other subjects had been assigned to particular groups on the basis of the values of the lipid factors in the fasting serum In the case of the control group it appeared that the glyceride glycerol level of serum both 4 hours and 7 hours after the fat meal was linearly related to the fasting value enabling estimation of either of the former values from the latter value Thus in the controls the serum glyceride-glycerol level 4 hours and 7 hours after the fat meal was respectively 1 65 and 1 1 times the corresponding level of fasting serum Only two groups of patients deviated from the first relationship One of these groups comprised males with clinical atherosclerosis normal serum cholesterol and serum glyceride-glycerol not exceeding 2 mMol/l The distribution of this group about the regression line for the controls was on a significantly higher level

Glyceride glycerol level of 4 hour serum equals 1 8 times the glyceride glycerol level of fasting serum up to about 4 mMol/l a limit above which the 4 hour values are less than 1 65 times the fasting value

In the control group the serum glyceride glycerol level 7 hours after the fat meal can be estimated at 1 17 times the fasting value Yet the rela

tion seems to hold only for glyceride-glycerol levels of fasting serum not exceeding mMol/l. Scattered glyceride-glycerol levels of fasting serum exceeding mMol/l were observed. 70 per cent of them lay above the line $y = 1.1x$ and the frequency of extreme 7 hour values also seems to be higher at fasting values over _ mMol/l. What this means is that the serum glyceride-glycerol values 4 and 7 hours after the fat meal are strongly associated with the fasting value but that the nature or strength of the association changes at different levels of the fasting value. So far as these associations are concerned, atherosclerotics did not differ from non-atherosclerotics with the same fasting serum glyceride-glycerol.

Similarly the 7 hour glyceride-glycerol values are associated with the 4-hour values in a manner that depends upon the level of the 4-hour value. Thus 4 hour serum glyceride-glycerol values exceeding 4 mMol/l tend to be accompanied by similar or higher 7 hour values whereas 4-hour values below 4 mMol/l usually yield an approximately 30 per cent lower 7 hour value. Nor in this respect did atherosclerotics deviate from non-atherosclerotics. On the other hand hyperlipaemics and hypercholesterolaemics differed from the controls in having proportionately higher 7 hour values in relation to the 4-hour values. Assuming that the controls as well as the hypercholesterolaemics and hyperlipaemics attain the maximum on their serum glyceride-glycerol curve 4 hours after the fat meal, then the rate of fat removal from the blood must be lower in the two latter groups than in the controls. The reason why so many investigators have reported differences between controls and atherosclerotics in this very removal phase after fat loading is presumably that selection of a group of atherosclerotics is tantamount to selection of subjects with hypercholesterolaemia and hyperlipaemia.

When the mean serum glyceride-glycerol levels immediately before as well as 4 and 7 hours after the fat meal are compared in different groups, allowance must be made for the interactions between the values. When such interactions are disregarded and groups are compared on the basis of respectively fasting 4-hour and 7-hour values, the result will be what most previous workers have done and which is correct for turbidity because it is not subject to such interaction. Various comparisons of this type disclose that the serum glyceride-glycerol level is far superior to the turbidity as a criterion for distinguishing between groups of patients. Furthermore in this respect the glyceride-glycerol level of fasting serum is superior to the corresponding levels of either 4 hour serum or 7 hour serum. Accordingly it seems rather pointless to carry out the fat tolerance test.

Serum and its subnatant. A further means of distinguishing between atherosclerotics and non atherosclerotics was probed. It constitutes the glyceride glycerol distribution in the chylomicron phase and subnatant of serum. It was

demonstrated that the fasting serum of the controls had a *chylomicron* phase unlike the other groups in which the serum glyceride-glycerol level had to exceed 4 mMol/l before the chylomicron phase became significant Conversely 4 hours after the fat meal all groups of subjects had a chylomicron phase regardless of the glyceride-glycerol level of whole serum and this chylomicron phase became proportionately larger the higher the whole-serum glyceride glycerol Here a difference became apparent between men and women — men have a higher proportion of their neutral fats in the form of chylomicrons than women No corresponding difference was apparent 7 hours after the fat meal No difference could be found between groups with and without clinical atherosclerosis in this respect

An association exists between the turbidity and glyceride-glycerol level of serum and it appeared that the nature of this association in non atherosclerotics was different from that in atherosclerotics This difference was significant for fasting serum But glyceride-glycerol of the chylomicron phase of the same serum was equally large in these two groups (the regressions of the subnatant upon whole serum were practically *identical as were the re*gressions of subnatant turbidity upon whole serum glyceride glycerol) The situation was similar for serum 4 hours after the fat meal but could not be demonstrated for serum 7 hours after the fat meal conceivably because 7 hour serum was obtained only from about one third of the number of subjects supplying fasting serum and *4-hour serum With respect to serum* immediately before and 4 hours after *the fat meal the conclusion may be* drawn that the glyceride glycerol content of the chylomicron *phase produces* different degrees of whole serum turbidity in non atherosclerotics and atherosclerotics in other words the chylomicrons are qualitatively different This suggests that there may exist chylomicrons which can be a factor in the development of atherosclerosis on account of their properties rather than their quantity

The investigations described in the foregoing were designed to reveal whether atherosclerotics differ from non atherosclerotics in some way susceptible to detection by quantitative *changes in serum glyceride-glycerol or* turbidity in response to a fat meal By and large the answer must be no The two exceptions however may have definite importance It should be restudied whether at every level of glyceride-glycerol a group with clinically proven atherosclerosis will have a less turbid serum than a group with no clinically detectable atherosclerosis The second suggestive finding that the clinically atherosclerotic *normocholesterolemic* and normoglyceridemic *group of males* had a significantly higher glyceride glycerol level at 4 hours than the average 1 65 times the fasting value is to some extent invalidated by the smallness of the group This also calls for further studies

Fat tolerance tests on different diet The fat tolerance of a few patients

was tested on a diet with predominantly saturated fats and then repeated on a diet with predominantly polyunsaturated fats. It appeared that the fasting glyceride-glycerol and turbidity values were approximately 40 per cent lower after the change and the response to the fat meal similar in principle but on a lower level. On both occasions the equations for the regression of 4 hour glyceride-glycerol upon fasting glyceride-glycerol were similar and so were the equations for the regression of the 7 hour values upon the fasting values

demonstrated that the fasting serum of the controls had a chylomicron phase unlike the other groups in which the serum glyceride-glycerol level had to exceed 4 mMol/l before the chylomicron phase became significant Conversely 4 hours after the fat meal all groups of subjects had a chylomicron phase regardless of the glyceride-glycerol level of whole serum and this chylomicron phase became proportionately larger the higher the whole-serum glyceride-glycerol Here a difference became apparent between men and women — men have a higher proportion of their neutral fats in the form of chylomicrons than women No corresponding difference was apparent 7 hours after the fat meal No difference could be found between groups with and without clinical atherosclerosis in this respect

An association exists between the turbidity and glyceride glycerol level of serum, and it appeared that the nature of this association in non atherosclerotics was different from that in atherosclerotics This difference was significant for fasting serum. But glyceride-glycerol of the chylomicron phase of the same serum was equally large in these two groups (the regressions of the subnatant upon whole serum were practically identical as were the regressions of subnatant turbidity upon whole serum glyceride glycerol) The situation was similar for serum 4 hours after the fat meal but could not be demonstrated for serum 7 hours after the fat meal conceivably because 7 hour serum was obtained only from about one-third of the number of subjects supplying fasting serum and 4-hour serum With respect to serum immediately before and 4 hours after the fat meal the conclusion may be drawn that the glyceride-glycerol content of the chylomicron phase produces different degrees of whole serum turbidity in non atherosclerotics and atherosclerotics in other words the chylomicrons are qualitatively different This suggests that there may exist chylomicrons which can be a factor in the development of atherosclerosis on account of their properties rather than their quantity

The investigations described in the foregoing were designed to reveal whether atherosclerotics differ from non atherosclerotics in some way susceptible to detection by quantitative changes in serum glyceride-glycerol or turbidity in response to a fat meal By and large the answer must be no The two exceptions however may have definite importance It should be restudied whether at every level of glyceride-glycerol a group with clinically proven atherosclerosis will have a less turbid serum than a group with no clinically detectable atherosclerosis The second suggestive finding that the clinically atherosclerotic normocholesterolemic and normoglyceridemic group of males had a significantly higher glyceride-glycerol level at 4 hours than the average 1 65 times the fasting value is to some extent invalidated by the smallness of the group. This also calls for further studies

Fat tolerance t[illegible] de on different d[illegible] The fat tolerance of a few patients

female). Only * of the other 0 groups differed significantly from the controls in this respect normocholesterolaemic normoglyceridaemic patients with clinical atherosclerosis having higher values and hyperlipaemic women without clinical atherosclerosis lower values than expected. The regression seems to be true up to glyceride-glycerol levels of 4 mMol/l for the other groups

For the association between glyceride-glycerol in control serum before (x) and 7 hours after the fat meal (y) the following regression was found. $\hat{y}$ = = 1 1 x 1 17 is not =1 i.e. the glyceride-glycerol level in serum 7 hours after the fat meal did not differ significantly from the fasting level. The spread of the observations about this line was very great and the statistical analysis showed that the total material from the fat tolerance point of view was tolerably homogenous and the association for the whole material could be expressed by the regression $\hat{y}$ = 1 44x The regression $\hat{y}$ = 1 17x is held to be true up to a serum level of mMol l No differences were shown between various groups without and with clinical atherosclerosis.

The association between glyceride-glycerol in serum 7 and 4 hours after the fat meal for levels below 4 mMol/l differed from that above this level. Below this level there was usually a decrease of 30 per cent and above it often an increase of glyceride-glycerol in serum between the 4th and th hour after the fat meal. No difference between groups without and with clinical atherosclerosis was shown but hyperlipaemics and hypercholesterolaemics differed from the controls in having proportionately higher hour values in relation to 4-hour values

No association was demonstrated between turbidity in serum before and 4 and 7 hours after the fat meal Almost all comparisons between the various hypercholesterolaemic groups and the group with normocholesterolaemic normoglyceridaemic atherosclerosis demonstrated no differences from the corresponding control group either in fasting serum turbidity or in 4 and hour values. Similar comparison revealed that glyceride-glycerol was superior for distinguishing clinical groups.

Glyceride-glycerol in the chylomicron phase did not differ in patients without and with clinical atherosclerosis either before or 4 and 7 hours after the fat meal. On the other hand there was a difference between men and women so that men had more chylomicron glyceride-glycerol than women 4 hours but not 7 hours after the fat meal. Controls had a small chylomicron phase in fasting but the other subjects showed no such phase below glyceride-glycerol levels of 4 mMol/l in fasting serum

Subjects without clinical atherosclerosis differed from those with atherosclerosis as to the turbidity in relation to glyceride-glycerol in serum before and 4 hours after the fat meal, patients with clinical atherosclerosis having lower turbidity than those without No difference was demonstrated be-

VI

GENERAL SUMMARY

The aim of this investigation was *in the first place to study the value* of the fat tolerance test in distinguishing subjects with and without atherosclerosis but also to compare the fat tolerance tests of an individual on ordinary diet with saturated *fat* and on a diet with poly unsaturated fat. The fat meal consisted of 200 ml 40% cream. Serum turbidity glyceride-glycerol cholesterol and phospholipids were determined before as well as 4 and 7 hours after the fat meal in a) controls (males and females) b) hypercholesterolaemics (male and females) c) hyperlipaemics (males and females) and d) normoglyceridaemic normocholesterolaemics with atherosclerosis (males only). Thus totally 11 groups were studied. Lipids and turbidity in serum at the fat tolerance test (before and 4 hours after the meal) were not associated with stature weight or body surface area. Hence the use of a standard fat meal was justified.

The reproducibility of the lipid and turbidity measurements before and 4 hours after the fat meal was studied mainly in hypercholesterolaemics and hyperlipaemics and only in a small group of normocholesterolaemic normoglyceridaemic subjects (others). Fasting glyceride-glycerol varied by *22—23 per cent in hyper*cholesterolaemics and in others while in hyperlipaemics it varied by 73 per cent. Corresponding values for turbidity were 80 64 and 235 per cent. Glyceride glycerol and turbidity in serum 4 hours after the fat meal were *so associated with corresponding values* in fasting serum that higher fasting values usually were accompanied by higher 4 hour values. If the relation between glyceride-glycerol in 4 hour serum and that in fasting serum was used as a measure of reproducibility of the fat tolerance test the "error of the method" was estimated to about 20 per cent.

Cholesterol in fasting serum at duplicate fat tolerance tests on unchanged diet varied by 11 per cent and phospholipids by 13 per cent. Cholesterol and phospholipid changes from fasting to 4 hour serum were greatest in that test where the fasting values were smallest. Serum cholesterol sometimes increased sometimes decreased.

For the clinical groups however association was shown only between glyceride-glycerol in serum before and 4 hour after the fat meal but not for turbidity cholesterol or phospholipids. The regression of glyceride glycerol in serum 4 hours after the fat meal (y) upon that of fasting serum (x) was $\hat{y} = 1.6x$ for the controls (male+

REFFRENCES

A[illegible], E. H. & K[illegible], M. D The stabilisation of serum lipid emulsions by serum phospholipids. J exp Med. 90: 409 1949.

Albrink, M. J Lipoprotein pattern as function of total triglyceride concentration of serum. J clin Invest. 40: 536, 1961

Albrink, M. J Ma[illegible] E B & Peters, J P The relation of neutral fat to lactescence of serum. J clin. Invest. 34 147 1954

Albrink, M. & Man, E. B Effect of carbohydrat ingestion on postprandial lipemia. Clin. Res Proc 4 121 1956.

Albrink, M. J & [illegible] R S: Effect of previous starvation on the response of plasma lipids and free fatty acids to fa meal J clin Invest. 3. 441 1960

Andersvall, G Fettbelastning vid hjärtinfarkt Nord. Med 64 1178 1960.

Bang I Ueber Lipämie I—II Biochem. Z 91 104 1918

Barritt D W Alimentary lipemia in men with coronary artery disease and in control Brit med. J 2 640, 1956.

Becker, G H Meyer, J & Necheles, H.: F t absorption and atherosclerosis. Science 110 529 1949

Becker, G H Meyer, J & Necheles, H Fat absorption in young and old age. Gastroenterology 14 87 1950.

Berkowitz D Likoff W & Spitzer, J J Serum triglyceride, unesterified fat y acid, and radioactiv fat clearance studies. Fed. Proc 18 191 1959.

Billimoria, J D Drysdale, J J[illegible], D C O & Maclagan N F Determination of fibrinolytic activity of whole blood ith special reference to the effect of exercise and fat feeding. Lancet. ii 471 1959

B[illegible] H J & H[illegible], H.: Undersøgelser over lipæmi II. Hospitalstidende. 67 29 1924

Blix G Studies on diabetic lipemia. Lund, AB PH. Lindstedts Univ Bokhandel 1925

Block, W J Barker, N W & Mann F D Effect of small doses of heparin in increasing the translucence of plasma during alimentary lipemia Circulation. 4 674 1951

Bloor, W R. Fat assimilation. J biol. Chem. 24: 447 1916.

Blotner H.: Blood fat tolerance tests in malnutrition and obesity Arch. intern. Med. 85 131 1933.

Boren E., Gene vot B & Holmberg H: Investigation of the daily rhythms in the production of chylomicrons in man on the supply of fat Acta med scand. 106: 570 1941

Bottcher, J A. D & Boorte-Stewart B.: Alimentary lipemia and ischemic heart disease Lancet 7173 363 1961

Boorte-Stewart B & Blackburn H. (1956). The effect of corn oil on lipid clearance in patients with ischaemic heart disease In Essential Fatty Acids ED H M Sinclair Butterworths (London), p. 186

Brown D F Heslin A. S. & Doyle, J T Postprandial lipemia in health and in ischaemic heart disease. A comparison of three indexes of fat absorption and removal and their modification by systemic heparin administration. New Engl. J Med. 264 733, 1961

Bruu G Changes in the lipid contents of serum in patients with manic — depressive psychoses Acta psychiat. Suppl. 22, 1940

Carlson L. A.: Determination of serum glycerides. Acta Soc Med. upsalien. 64 208, 1959.

Carlson L. A.: Serum lipids in normal men. Acta med. scand. 167 377 1960.

tween glyceride glycerol in the chylomicron phase of these groups so there must be a difference in chylomicron size (smaller in patients with atherosclerosis)

When lipids and turbidity of serum were lowered by changing the diet from mainly saturated fats to mainly unsaturated fats a fat meal repeated on the latter diet showed fundamentally the same course but on a lower level

ACKNOWLEDGMENTS

Throughout this investigation I have been much encouraged and stimulated by my colleague and friend Associate Professor BERTIL HOOD M D I thank him deeply I am also most grateful to the chief of Medical Department I Professor LARS WERKÖ M. D for continuous generous support and for making available laboratory facilities My work has been greatly facilitated by my chief Assistant Professor TORSTEN LINDQVIST M. D to whom I am much indebted Assistant Professor LENNART ANGERVALL, M D constructively criticized my work and merits my sincere thanks Mr ESBJÖRN CARLSTROM M A rendered invaluable help with the statistical work. FOLKE HEYMAN M D assisted me with the interpretation of electrocardiograms.

I have pleasure in acknowledging my indebtedness to the ward nurses especially KERSTIN AHLQUIST INGA HWASS ETHEL HOGBERG Technical and clerical assistance was capably rendered by INGRID KARLSSON GUNNEL SVENSSON MARIANNE AHLSTRÖM and KAJSA ANGERVALL.

The investigation was supported by grants from the Swedish National Association Against Heart and Chest Diseases

KUO, P. T., JOYNER, C. R. & REINHOLD, J. G. Effects of fat ingestion and heparin administration on serum lipids of "Normal" hypercholesterolemia, hyperlipemia and atherosclerotic subjects. Amer. J. med. Sci. 235: 613, 1958.

MALMCRONA, R.: Totalt serumcholesterol och cholesterol i elektroforetiskt skilda alfa — och — betalipoproteinfraktioner i ett normalmaterial. Nord. Med. 64 1187 1960

MAN, E. B. & ALBRINK, M. J. Serum lipids in different phases of carbohydrate metabolism. Yale J. Biol. Med. 29: 316, 1956.

MAN, E. B. & GILDEA, E. F. The effect of ingestion of a large amount of fat and of a balanced meal on the blood lipids of normal man. J. biol. Chem. 99 61 1932.

MARDER, L., BECKER, G. H., MAIZEL, B. & NECHELES, H.: Fat absorption and chylomicronemia. Gastroenterology 20: 43, 1952.

MEERWOLK, W. J. & POMOSOWSKA, Y. Influence of small doses of heparin on fat tolerance curves in vivo and in vitro. Proc. Soc. exp. Biol. 83: 164 1953.

MITCHELL, J. R. A. & BRONTE-STEWART, B. Alimentary lipaemia and heparin clearing in ischaemic heart-disease. Lancet 7083: 167 1959

MORETON, J. R.. Atherosclerosis and alimentary hyperlipemia. Science 106: 190, 1947

MORETON, J. R.. Physical state of lipids and foreign substances producing atherosclerosis. Science 107 371 1948.

MORETON, J. R.. Chylomicronemia, fat tolerance and atherosclerosis. J. Lab. clin. Med. 35: 373, 1950.

NESTEL, P. J., DENBOROUGH, M. A. & O'DEA, J. Disposal of human chylomicrons administered intravenously in ischaemic heart disease and essential hyperlipemia. Circulat. Res. 10: 786, 1962.

NEUMANN, A.: Ueber ultramikroskopische Blutuntersuchungen zur Zeit der Fettresorption bei Gesunden und Kranken. Wien. klin. Wschr. 20: 851 1907

NIKKILA, E. A. & KONTTINEN, A.: Effect of physical activity on postprandial levels of fats in serum. Lancet. 1151 1962.

NYMARK, M. L.: Beitrag zur Betrachtung der alimentären Lipemie des Menschen. Acta med. scand. 74 466, 1931

OSHAW, K. L., ZINN, W. J. & WHARTON, G. K.: Comparison of serum turbidity chylomicronemia and total lipid values after fat test meal. J. Amer. med. Ass. 164: 633 1957

POMERANZE, J., BEINFIELD, W. H. & CHESSIN, M.: Serum lipid and fat tolerance studies in normal, obese and atherosclerotic subjects. Circulation, 10: 742, 1954

SCHWARTZ, L., WOLDOW, A. & DUNSMORE, R. A.: Determination of fat tolerance in patients with myocardial infarction. J. Amer. med. Ass. 149: 364 1952.

SEIDER, H. & HAUCK, W. Zur Frage der medikamentösen Beeinflussung der Serumlipide nach alimentärer Fettbelastung. Z. ges. inn. Med. 18 348, 1963

SNEDECOR, G. W. Statistical Methods. Applied to experiments in agriculture and biology 5th ed. Ames. Iowa. 1956.

SPERRY, W. M. & SCHOENHEIMER, R.: A comparison of serum, heparinized plasma, and oxalated plasma in regard to cholesterol content. J. biol. Chem. 110: 655, 1935.

SPERRY, W. M. & WEBB, M.: An revision of the Schoenheimer — Sperry method for cholesterol determination. J. biol. Chem. 187 97 1950.

SPERRY, W. M. & WEBB, M. A.: A revision of the Schoenheimer-Sperry method for cholesterol determination. J. biol. Chem. 187 97 1950.

SULLIVAN, J. F. The effect of protein ingestion on alimentary lipemia. Amer. J. med. Sci. 243: 770, 1962.

SVANBORG, A. & SVENNERHOLM, L.: Determination of unesterified fatty acids in normal human plasma. Clin. chim. Acta. 3: 443, 1958.

SVANBORG, A., SVENNERHOLM, L.: Plasma total lipid, cholesterol, triglycerides, phospholipids and free fatty acids in healthy scandinavian population. Acta med. scand. 169: 43, 1961

SWANK, R. L. & LEVY, R. W. Chylomicron dissolution dosage and size of action of heparin. Am. J. Physiol. 171 208, 1952.

CARLSON L. A. & WADSTRÖM L. B.: On the occurrence of tri-di and monoglycerides in human serum Third Int. Conf on Biochemical Problems f Lipids. Brussels 1956 p. 123

CARLSON L. A. & WADSTRÖM L. B.: Determination of glycerides in blood serum. 4 1 1959

COHEN H & GOLDBERG C.: Effect of physical exercise on alimentary lipaemia. Brit. med J 2 509 1960.

CRAMÉR K.: Serum β lipoprotein lipids and protein in normal subjects of different sex and age Acta med scand 171: 413 196..

CRAMÉR, K. & ISAKSSON B An evaluation of the Theorell method for the determination f total serum cholesterol Scand J clin Lab Invest. 11 13 1959

DAMBOROUGH, M A Aliment ry l pemia in ischaemic heart disease Clin. Sci 25: 115 1963

McDONALD G A. & FULLERTON H W.: Effect of phenindion and bed rest on blood coagulability following a high f t int ke Lancet, ii 1111 1960

ECGSTEIN M & SCHETTLER, G The ffect of feeding various f t n the levels of blood lipids. Proceedings f F rth International Conference on the Biochemical Problems of Lipids. Essential F tty Acids. 111 1958

FRAZER A C & STEWART H C Ultra microscopic particles in normal human blood J Physiol (Lond) 90 16 1937

GAGE, S H & FISH P A F t digestion, bsorption, and ssim lation man and animals as determined by the la k field microscope and a fatsol ble dye Amer J Anat 34 1 1924

GROOVER, M. E. JR. JERNI AN J A and MARTIN C D V riation in serum l pid concentration and linical coronary disease Amer J med Sc 55 133 1960

GRU NER A & HILDEN T The occurrence f hylomicrons in the blood young and old individuals. Scand. J lin Lab In est 5: 236 1953

HAVEL, R.: Early effects of f t ingest on on l pids and l poproteins of serum in ma J clin Invest. 6 848 195

HERNSTEIN J., WANG C ADLER ERG D Fat loading studies in relation to age Circulation 8 450 1953.

HILLER, A LINDER G C LUNDSGAARD C. & VAN SLYKE D D.: F t met bolism in nephritis. J exp Med 39 931 1924

HIRSCH E F & CARBONARO L. Serum esterified fatty acids with f t tolerance tests in Diabetes mellitus. Arch v I t Med 86 519 1950.

HOOD B & ANGERVALL, G The relation be tween inhibition towards clearing total cholesterol nd distribution of cholesterol in plasma. Acta med scand 118. 12 1957

HOOD B & ANGERVALL, G: Studies in essential hypercholesterolemia nd xanthomatosis. Relationsl ips between ge sex, cholesterol concentration in plasma fractions and size of tendious deposits. Circulation. 26 30 1959

HORLICK, L.: The effect of a test meal f animal fat on the lipid and lipoproteins of h man serum, and some aspect of liver function in normal individuals and in those with coronary therosclerosis. Circulation 14 409 1956.

HORLICK, L. Effect of acut fat load on serum lipid in atherosclerosis. Circulat Res 4 368, 1957

IDU S M. POPESCO P COCIUMIAN & THEODORESCO B La valeur de l épreve du repa gras dan l thérosclérose avec normo ou hypercholestérolémie Sem Hôp. 38 3903 1962

JONES, R. J & DOBRILO I Factors in fluencing f t tolerance curve Circulation 28 662, 1963

KINGSBURY K J & MORGAN D M Basic types of lipid response Clin Sc 19 645 1960

KINGSBURY K. J MORGAN D M & SHERRINGTON P C The effect of test feed on the plasma lipids. Lancet 1015, 1960.

KONTTINEN A RAJASALMI M. Effect of heavy cigarett smoking on postprandial triglycerides, free f tt acids, and cholesterol. Brit. med. J 1 850 1963

K o P T & JOYNER, C. R. Angina pectoris nduced by fat ingestion in patient with coronary artery disease J Amer med Ass. 1 5 1008 1953

TALBOTT G D & KEATING B M: Effects of preprandial whiskey on postalimentary lipemia. Geriatrics. 17: 802, 196..

TANNHAUSER, S. J.: *Serum lipids and their value in diagnoses.* New Engl J Med. 237 515 1947

THEORELL, H & WIDSTRÖM, G: Zur Methodik der Lipoidanalysen im Blut unter besonderer Berücksichtigung des Gesamt-Cholesterins. Zschr ges exper Med. 75: 699 1931

THEORELL, H.. Ueber quantitative Bestimmung der Lipoide der aus Pferdeplasma ausgesalzten Eiweisskörper Biochem. Z. 175: 296 1926

VAN HANDEL, E., & ZILVERSMIT D B *Micromethod for the direct determination of* serum triglycerides. J Lab Med. 50-15., 1957

WOLDOW A., CHAPMAN J E., EVAN J M: Fat tolerance in subjects with atherosclerosis. Heparin effects upon lipemia, lipoproteins, and gammaglobulins. Amer Heart. J 47 568, 1954.

ACTA MEDICA SCANDINAVICA

SUPPLEMENTUM 423

AN ATROPHIC CIRCUMSCRIBED SKIN LESION IN THE LOWER EXTREMITIES OF DIABETICS

by

HANS MELIN

Accompanies Vol. 176

UMEÅ 1964

GÖTEBORG 1964
ELANDERS BOKTRYCKERI AKTIEBOLAG

ACTA MEDICA SCANDINAVICA

SUPPLEMENTUM 428

AN ATROPHIC CIRCUMSCRIBED SKIN LESION IN THE LOWER EXTREMITIES OF DIABETICS

by

HANS MELIN

Accompanies Vol. 176

UMEÅ 1964

GÖTEBORG 1964
ELANDERS BOKTRYCKERI AKTIEBOLAG

ACTA MEDICA SCANDINAVICA

SUPPLEMENTUM 423

AN ATROPHIC CIRCUMSCRIBED SKIN LESION IN THE LOWER EXTREMITIES OF DIABETICS

by

HANS MELIN

Accompanies Vol. 176

UMEÅ 1964

GÖTEBORG 1964
ELANDERS BOKTRYCKERI AKTIEBOLAG

FROM THE DEPARTMENT OF MEDICINE
(HEAD PROFESSOR NILS TORNBLOM) UNIVERSITY OF UMEÅ, SWEDEN

An atrophic circumscribed skin lesion in the lower extremities of diabetics

by

HANS MELIN

ALSO PUBLISHED AS SUPPLEMENT 423 TO
ATCA MEDICA SCANDINAVICA

UMEÅ 1964

ACTA MEDICA SCANDINAVICA

has been published since *1919* as a continuation of Nordiskt Medicinskt Arkiv founded in 1869 by Axel Key The first volume of Acta Medica Scandinavica *is therefore numbered LII (52)*

The chief editors have been Axel Key 1869—1900 C. G Santesson 1901—1915 I Holmgren 1916—1957 and Birger Strandell 1958 to date.

Acta Medica Scandinavica publishes original research papers in the field of internal medicine. Priority will be given to authors from countries which are represented on the editorial board Contributors from those countries should submit their papers to a representative of the country in question. Contributors from other countries should send their papers to the Editor at the address below

Submission of a manuscript for publication will be held to imply that the paper has not been published elsewhere and that, if accepted it will not be republished in any other journal in the same or similar form without the Editor s written consent

Papers will be published in English French or German at the authors choice, *followed by a summary in English.*

The manuscripts shall be in final form as submitted. They shall be typewritten with double spacing and broad left hand margin

If a paper exceeds 16 printed pages, the cost for the excess pages shall be borne by the author No paper shall exceed 24 printed pages.

Subscription

The annual subscription to the journal, covering two volumes, each of 6 numbers, is 140 Sw crowns or U.S *$ 27 25 including postage* in the Scandinavian countries and in Holland 120 Sw crowns

Address for subscriptions and all communications
ACTA MEDICA SCANDINAVICA
P O Box 2052 Stockholm 2

Requests for duplicate copies of numbers that *have gone astray can only be* considered if made within a *fortnight of the receipt of the following number*

FROM THE DEPARTMENT OF MEDICINE
(HEAD PROFESSOR NILS TÖRNBLOM) UNIVERSITY OF UMEÅ, SWEDEN

An atrophic circumscribed skin lesion in the lower extremities of diabetics

by

HANS MELIN

ALSO PUBLISHED AS SUPPLEMENT 423 TO
ATCA MEDICA SCANDINAVICA

UMEÅ 1964

TRANSLATED BY

FREDERICK A. L. CHARLESWORTH

[illegible]ker aktiebolaget City — Umeå 1964

To my wife

TRANSLATED BY

FREDERICK A. L. CHARLESWORTH

Tryckeriaktiebolaget City — *Umeå 1964*

PREFACE

The incentive to undertake this investigation came from my Chief Professor Nils Thyresson, who first noticed the atrophic, circumscribed skin lesions and their association with diabetes and praediabetes.

PREFACE

The incentive to undertake this investigation came from my Chief Professor Nils Törnblom, who *first* noticed the atrophic, circumscribed skin lesions and their association with diabetes and praediabetes.

CONTENTS

CHAPTER I

VASCULAR DISEASE IN DIABETES

The course and prognosis of diabetic disease to-day is practically dominated by vascular changes. Insulin brought about a radical change in the course of the disease and prolonged the span of life. Formerly diabetic coma was a common cause of death, whereas to day it is unusual that a diabetic succumbs to it. Instead it is the vascular changes and their kindred changes that prove decisive for the course of the disease, and resultant mortality thereof is much greater among diabetics in comparison with non-diabetics (Bell 1952). They also result in a more or less progressive invalidity that is accompanied by constant changes in the patient's social status.

Vascular changes in diabetes are partly located in the larger vessels, particularly in the coronary arteries and the arterial vessels of the legs, and partly in the small vessels e. g. the arterioles, the capillaries and the venules. The changes in the larger vessels are in congruence to those seen in arteriosclerosis and qualitatively cannot with certainty be separated from them. The changes in the small vessels are of an entirely different type and are often characteristic, if not specific, for diabetic disease. The small vessel disease in diabetes has, during the past two decades attracted more and more attention and it has even been emphasized as to whether it contributes to the arteriosclerosis in the larger vessels by affecting the vasa vasorum (cf Warren and Le Compte 1952)

The changes in the small vessels have primarily been found in the eyes and kidneys. There is, however more and more proof for the supposition of the occurrence of a more or less generalized diabetic angiopathy (cf Lundbaek 1949 1960) As a rule, diabetic disease is diagnosed several years prior to the vascular changes being clinically manifest. There is a pronounced positive correlation between the duration of diabetes and the frequence of retinopathy and nephropathy A corresponding condition for the arteriosclerotic changes in the larger vessels is not so obvious. The latter appear to be more correlated to the patient's age (Lundbaek 1953 Liebow et al. 1955) The arteriosclerotic changes are considered to arise earlier and are more quantitatively pronounced than in corresponding age groups of non-diabetics (Lisa et al. 1942 Bell 1952). This has, however been repudiated in works by Goldenberg et al. (1958) and Blumenthal et al. (1960) They doubt if there is any difference in the intensity of arteriosclerosis in the aorta and the larger arteries in the extremities between diabetics and non-diabetics when the factors of age, sex and hypertension are properly assessed. Instead, they assert that there are

proliferative changes in the smaller vessels and find on an average that these changes are two and a half times more frequent in diabetics than in non-diabetics.

The histopathological changes in the small vessels appear best with PAS-staining (periodic acid Schiff) which suggests that they consist of mucoproteins and neutral mucopolysaccharides (McManus 1948) Through a direct chemical analysis of hyalinized glomeruli isolated from diabetic human kidneys with Kimmelstiel Wilson changes Odin and Tornblom (1959) found no difference in this respect between diabetic and non-diabetic glomeruli The amount was the same as in normal serum In contradistinction to normal glomeruli the hyalinized glomeruli contained blood protein

A brief survey will be given in the following of the most common diabetic complications considered to be connected with diabetic angiopathy

DIABETIC RETINOPATHY

Diabetic retinopathy is often an early clinical indication of diabetic angiopathy Diabetic retinopathy was first discribed by MacKenzie in 1877 and by Nettleship in 1888 They proved that the anatomic substratum of the small punctuated red dots seen at the ophthalmologic examination consisted of microaneurysms. The latter were rediscovered by Ballantyne in 1943 They could be demonstrated through the so-called flat preparation of the retina and by filling the retinal vessels with Indian ink, or by PAS-staining They are located in the capillaries — chiefly to their venous parts. The wall of the aneurysm often reveals a pronounced hyaline thickening, sometimes with endothelial proliferations and thrombotic formation (Ballantyne and Loewenstein 1944 Ballantyne 1945).

The genesis of the aneurysms is unknown as is that of diabetic angiopathy in its entirety The similarity with the capillary changes in the glomeruli in diabetic nephropathy has been pointed out (Ashton 1949) Diabetic retinopathy has certain similarities with a number of other eye diseases where tissue anoxia occurs with subsequent neovascularization (Wise 1956) Examples of such conditions are central vein thrombosis, periphlebitis retinae malignant hypertension and sickle cell anaemia. Indications of microaneurysms have also been found in these conditions (Ballantyne and Loewenstein 1944 Wexler and Branover 1950) Microaneurysms have sometimes been found in entirely healthy eyes. They were however considerably less and situated in the periphery in contradistinction to diabetic retinopathy where they are larger and partial to the macular area (Ashton 1951) A dilatation of the retinal veins is also seen in the ophthalmoscopic picture At later stages there is an increase in the number of aneurysms and the occurrence of deep hemorrhages and exudates. In the most advanced stadium the so-called proliferative stadium there is a new formation of vessels and connective tissue with preretinal hemorrhages and bleeding in the corpus vitreum.

Diabetic retinopathy may gradually lead to blindness and the prolonged duration of the disease has increased the frequence of blind diabetics With a dia

betic duration of 20 to 30 years 10 to 15 per cent are blind (Lundbaek 1959 Winter 1960)

In rare cases a reddish discolouration is seen in the iris, rubeosis iridis. It is caused by a network of fine vessels on the frontal surface of the iris, the vessels probably being newly formed. Glaucoma often occurs in connection with rubeosis. In those cases where the eye-ground could be inspected a pronounced diabetic retinopathy is observed (Salus 1928 Kurz 1937) The incidence of rubeosis together with retinopathy indicates that rubeosis is conceived to be a diabetic vascular disease.

In a biomicroscopic study Ditzel (1954 1962) demonstrated changes in the bulbar conjunctival vessels in diabetics and above all a diminished relationship between the diameter of the arterioles and venules (A/V — ratio) of the larger pairs of vessels. He found that the changes appeared to be correlated to the duration of diabetes and that they also seemed to be more frequent in diabetics with retinopathy and nephropathy than in those without these complications. It was considered that the conjunctival vessel changes formed part of a specific diabetic angiopathy The correlation to the diabetic duration and to the incidence of retinopathy and nephropathy has not, with certainty however been confirmed (Bech et al. 1960).

NEPHROPATHY

The expression diabetic nephropathy is often used to denote chronic kidney changes of vascular or mixed vascular and infectious geneses in diabetics. The vascular changes are partly arterio- arteriolosclerotic and partly capillary The capillary changes were described by Kimmelstiel and Wilson (1936) who named them intercapillary glomerulosclerosis. It consists of a diffuse or a nodular form with hyalinization. The changes are stained with PAS (McManus 1948) The nodular form is generally considered specific for diabetes (Warren and Le Compte 1952 Bell 1953). Electronmicroscopic examinations have later supported the conception that the changes are primarily intracapillary (Bergstrand and Bucht 1957 Farquhar et al. 1959 Sabour et al. 1962)

Kimmelstiel and Wilson also reported a clinical syndrome characteristic of the so-called intercapillary glomerulosclerosis viz. the incidence of hypertension, massive proteinuria and oedema in diabetics. As a rule, the clinical picture is not so distinct, one or more of the symptoms may be lacking despite the characteristic patho-anatomical changes being pronounced. In general nephropathy is associated with a more or less pronounced albuminuria and slight hypertonia and gradually leads to renal insufficiency with uremia. Nephropathy is always associated with retinopathy and similarly reveals a positive correlation to the diabetic duration (Goodof 1945 Ashton 1949 Wilson et al. 1951)

NEUROPATHY

In diabetes changes often occur in the peripheral nerves of the type of *polyneuritis*. In recent years more and more support has been given to the supposition that this is caused by changes in the

proliferative changes in the smaller vessels and find, on an average, that these changes are two and a half times more frequent in diabetics than in non-diabetics.

The histopathological changes in the small vessels appear best with PAS-staining (periodic acid Schiff) which suggests that they consist of mucoproteins and neutral mucopolysaccharides (McManus 1948) Through a direct chemical analysis of hyalinized glomeruli isolated from diabetic human kidneys with Kimmelstiel-Wilson changes Odin and Törnblom (1959) found no difference in this respect between diabetic and non-diabetic glomeruli. The amount was the same as in normal serum In contradistinction to normal glomeruli the hyalinized glomeruli contained blood protein.

A brief survey will be given in the following of the most common diabetic complications considered to be connected with diabetic angiopathy

DIABETIC RETINOPATHY

Diabetic retinopathy is often an early clinical indication of diabetic angiopathy Diabetic retinopathy was first discribed by MacKenzie in 1877 and by Nettleship in 1888 They proved that the anatomic substratum of the small punctuated red dots seen at the ophthalmologic examination consisted of microaneurysms. The latter were rediscovered by Ballantyne in 1943 They could be demonstrated through the so-called flat preparation of the retina and by filling the retinal vessels with Indian ink or by PAS-staining They are located in the capillaries — chiefly to their venous parts. The wall of the aneurysm often reveals a pronounced hyaline thickening sometimes with endothelial proliferations and thrombotic formation (Ballantyne and Loewenstein 1944 Ballantyne 1945)

The genesis of the aneurysms is unknown as is that of diabetic angiopathy in its entirety The similarity with the capillary changes in the glomeruli in diabetic nephropathy has been pointed out (Ashton 1949) Diabetic retinopathy has certain similarities with a number of other eye diseases where tissue anoxia occurs with subsequent neovascularization (Wise 1956) Examples of such conditions are central vein thrombosis, periphlebitis retinae malignant hypertension and sickle cell anaemia Indications of microaneurysms have also been found in these conditions (Ballantyne and Loewenstein 1944 Wexler and Branover 1950) Microaneurysms have sometimes been found in entirely healthy eyes. They were, however considerably less and situated in the periphery in contradistinction to diabetic retinopathy where they are larger and partial to the macular area (Ashton 1951) A dilatation of the retinal veins is also seen in the ophthalmoscopic picture. At later stages there is an increase in the number of aneurysms and the occurrence of deep hemorrhages and exudates. In the most advanced stadium the so-called proliferative stadium, there is a new formation of vessels and connective tissue with preretinal hemorrhages and bleeding in the corpus vitreum.

Diabetic retinopathy may gradually lead to blindness and the prolonged duration of the disease has increased the frequence of blind diabetics With a dia

betic duration of 20 to 30 years 10 to 15 per cent are blind (Lundbaek 1959 Winter 1960).

In rare cases a reddish discolouration is seen in the iris, rubeosis iridis. It is caused by a network of fine vessels on the frontal surface of the iris, the vessels probably being newly formed. Glaucoma often occurs in connection with rubeosis. In those cases where the eye-ground could be inspected a pronounced diabetic retinopathy is observed (Salus 1928 Kurz 1937). The incidence of rubeosis together with retinopathy indicates that rubeosis is concerned to be a diabetic vascular disease.

In biomicroscopic study Ditzel (1954 1962) demonstrated changes in the bulbar conjunctival vessels in diabetics and above all a diminished relationship between the diameter of the arterioles and venules (A/V — ratio) of the larger parts of vessels. He found that the changes appeared to be correlated to the duration of diabetes and that they also seemed to be more frequent in diabetics with retinopathy and nephropathy than in those without these complications. It was considered that the conjunctival vessel changes formed part of a specific diabetic angiopathy The correlation to the diabetic duration and to the incidence of retinopathy and nephropathy has not, with certainty however been confirmed (Bech et al. 1960).

NEPHROPATHY

The expression diabetic nephropathy is often used to denote chronic kidney changes of vascular or mixed vascular and infectious geneses in diabetics. The vascular changes are partly arterio- arteriolosclerotic and partly capillary The capillary changes were described by Kimmelstiel and Wilson (1936) who named them intercapillary glomerulosclerosis. It consists of a diffuse or a nodular form with hyalinization. The changes are stained with PAS (McManus 1948). The nodular form is generally considered specific for diabetes (Warren and Le Compte 1952, Bell 1953). Electronmicroscopic examinations have later supported the conception that the changes are primarily intracapillary (Bergstrand and Bucht 1957 Farquhar et al. 1959 Sabour et al. 1962).

Kimmelstiel and Wilson also reported a clinical syndrome characteristic of the so-called intercapillary glomerulosclerosis viz. the incidence of hypertension, massive proteinuria and oedema in diabetics. As a rule the clinical picture is not so distinct, one or more of the symptoms may be lacking despite the characteristic patho-anatomical changes being pronounced. In general nephropathy is associated with a more or less pronounced albuminuria and slight hypertonia and gradually leads to renal insufficiency with uremia. Nephropathy is always associated with retinopathy and similarly reveals a positive correlation to the diabetic duration (Goodof 1945 Ashton 1949 Wilson et al. 1951).

NEUROPATHY

In diabetes changes often occur in the peripheral nerves of the type of polyneuritis. In recent years more and more support has been given to the supposition that this is caused by changes in the

nutritive vessels vasa nervorum and *consequently* secondary to diabetic angiopathy (Woltman and Wilder 1929 Fagerberg 1959 Goldenberg et al. 1959)

As a rule a distinction is made between an acute reversible form that occurs in connection with severe ketoacidosis and a long term diabetic form that is not reversible It is the latter which in the first place is usually denoted as diabetic neuropathy and which eventually is secondary to the changes in the vasa nervorum It is often symmetric and located in the lower extremities There may be a complete lack of symptoms or they may be dissimilarly notably pronounced. They often consist of paraesthesia or pains in the legs. The paraesthesia is experinced as tingling or burning and pains of a cramplike nature often occur at night In severe cases lightning pains may occur Neuropathy may be demonstrated in different degrees of severity In the milder cases are found absent achilles reflexes diminished or normal patella reflexes or a slightly impaired sense of vibration In the more severe cases are found absent achilles and patella reflexes a considerably reduced or absent sense of vibration and other disturbances in sensation and sometimes paresis

Impaired autonomic function sometimes accompanies the neuropathy and the most common signs are nocturnal diarrhea orthostatic hypotension pupil lotonia deficient perspiration bladder atony and loss of potency (Goodman et al 1953 Martin 1953 Aagenaes 1962) A moderate elevated spinal fluid protein content without pleocytosis is often demonstrated An increase of protein in the spinal fluid may also be proved in diabetics without neuropathy but with vascular complications The protein increase is presumed to be due to a change of the blood-brain barrier in all probability caused by diabetic vascular changes (Kutt et al 1961)

The incidence of diabetic neuropathy varies considerably from 5 per cent (Rundles 1945) to 65 per cent (Root 1959) according to the composition of the material and the diagnostic criteria chosen Diabetic neuropathy appears to be correlated to the diabetic duration and to the incidence of retinopathy and nephropathy (cf Fagerberg 1959 Aagenaes 1962 Steiness 1963)

Particularly in elderly badly controlled diabetics there sometimes occur bilateral asymmetric pareses with atrophy of the thigh musculature, especially of the quadriceps and pelvic girdle musculature d i a b e t i c a m y o t r o p h y The condition develops during the course of some weeks and is associated with severe pain in the affected regions The genesis is far from clear and is probably an expression of a metabolic disturbance of myogenic nature, or a combination of myogenic and neurogenic injury (Bischoff 1959 Locke et al 1963) In muscle biopsies from patients with this disease endothelial proliferations in the small vessels have recently been demonstrated (Gårde and Kugelberg 1963)

CORONARY DISEASE

Coronary sclerosis and coronary thrombosis occur considerably more among diabetics than among non-diabetics. In a very exhaustive study of the incidence

of coronary artery disease from about 50.000 autopsies Clawson and Bell (1949) found that fatal coronary disease was about twice as frequent in diabetic as in non-diabetic males and three times as frequent in diabetic as in non-diabetic females. In agreement with other authors they also found that the incidence of disease of the coronary arteries in diabetic women was almost as high as in diabetic men, in contrast to the marked preponderance of the disease in non-diabetic men over non-diabetic women (Lundbaek 1953 Bradley and Bryfolge 1956)

The mortality after a coronary occlusion is also greater among diabetics than among non-diabetics (Clawson and Bell 1949) Coronary disease appears to be correlated to age as well as to diabetic duration (Bryfolge and Bradley 1957) Coronary vascular changes in diabetics might possibly be a combination of atherosclerosis in the larger vessels and for diabetes more or less specific changes in the small vessels (Warren and Le Compte 1952, Lundbaek 1960) Blumenthal et al. (1960) also found proliferative PAS-positive changes in the intramural coronary vessels diabetics revealed such lesions about two and a half times as frequently as non-diabetics. The highest frequency of proliferative lesions was found in the medium sized arteries. The lesions were histochemically similar to those seen in diabetic glomerulosclerosis and they did not find common arteriosclerotic lesions more frequently among diabetics than among non-diabetics.

ARTERIAL DISEASE IN THE LOWER EXTREMITIES

It is evident that gangrene in the lower extremities is considerably more common in diabetics than in non-diabetics (Dry and Hines 1941 Bell 1950) Bell (1957) found that in patients over 40 years of age gangrene was about 50 times more common in diabetics than in non-diabetics, and that the ratio diabetic males to diabetic females was 1 to 1 in contrast to non-diabetics where it was 3 to 2. In Dry and Hines (1941) extensive series the same ratio for diabetic and non-diabetic men and women was 2 to 1 and 7 to 1 respectively Diabetic gangrene has also other characteristics that differentiate it from the non-diabetic. It is often located in one or more toes, or is patchily distributed over the foot. The pulsations of the foot arteries are often preserved, and in many instances the prognosis may be good. The relation between the arterial disease in the lower extremities and diabetic duration is not as conspicuous as in those vascular changes previously mentioned e g. the retinal and glomerular ones (Oakly et al. 1956 Bell 1957) There is a tendency to conceive the arterial disease in the lower extremities as a long-term diabetic symptom and as a combination of arteriosclerosis in the large and medium sized arteries and changes in the small vessels (Lundbaek 1960) Goldenberg et al. (1959) also support the idea of the incidence of a special diabetic angiopathy Similarly to the findings in the coronary arteries they described endothelial proliferation and the deposit of a PAS-positive material in the small arteries and arterioles studied in amputated specimens

nutritive vessels, vasa nervorum and consequently secondary to diabetic angiopathy (Woltman and Wilder 1929 Fagerberg 1959 Goldenberg et al 1959)

As a rule, a distinction is made between an acute reversible form that occurs in connection with severe ketoacidosis and a long term diabetic form that is not reversible It is the latter which in the first place, is usually denoted as diabetic neuropathy and which eventually is secondary to the changes in the vasa nervorum It is often symmetric and located in the lower extremities There may be a complete lack of symptoms or they may be dissimilarly notably pronounced. They often consist of paraesthesia or pains in the legs. The paraesthesia is experinced as tingling or burning and pains of a cramplike nature often occur at night In severe cases lightning pains may occur Neuropathy may be demonstrated in different degrees of severity In the milder cases are found absent achilles reflexes diminished or normal patella reflexes or a slightly impaired sense of vibration In the more severe cases are found absent achilles and patella reflexes, a considerably reduced or absent sense of vibration and other disturbances in sensation and sometimes paresis.

Impaired autonomic function sometimes accompanies the neuropathy and the most common signs are nocturnal diarrhea orthostatic hypotension pupillotonia, deficient perspiration bladder atony and loss of potency (Goodman et al 1953 Martin 1953 Aagenaes 1962) A moderate elevated spinal fluid protein content without pleocytosis is often demonstrated. An increase of protein in the spinal fluid may also be proved in diabetics without neuropathy but with vascular complications The protein increase is presumed to be due to a change of the blood-brain barrier in all probability caused by diabetic vascular changes (Kutt et al 1961)

The incidence of diabetic neuropathy varies considerably from 5 per cent (Rundles 1945) to 65 per cent (Root 1959) according to the composition of the material and the diagnostic criteria chosen. Diabetic neuropathy appears to be correlated to the diabetic duration and to the incidence of retinopathy and nephropathy (cf Fagerberg 1959 Aagenaes 1962 Steiness 1963)

Particularly in elderly badly controlled diabetics there sometimes occur bilateral asymmetric pareses with atrophy of the thigh musculature especially of the quadriceps and pelvic girdle musculature d i a b e t i c a m y o t r o p h y The condition develops during the course of some weeks and is associated with severe pain in the affected regions. The genesis is far from clear and is probably an expression of a metabolic disturbance of myogenic nature, or a combination of myogenic and neurogenic injury (Bischoff 1959 Locke et al 1965) In muscle biopsies from patients with this disease endothelial proliferations in the small vessels have recently been demonstrated (Gårde and Kugelberg 1963)

CORONARY DISEASE

Coronary sclerosis and coronary thrombosis occur considerably more among diabetics than among non-diabetics In a very exhaustive study of the incidence

CHAPTER II

DERMATOLOGIC LESIONS ASSOCIATED WITH DIABETES

There is a heterogeneous group of skin lesions that may occur in connection with diabetes. Although none of them is absolutely pathognomonic of diabetes they arise more often in diabetics than in non-diabetics. Among those skin changes generally considered to be diabetic are necrobiosis lipoidica diabeticorum and xanthoma diabeticorum.

Another group consists of bacterial and mycotic skin infections. They are considered to be more common among diabetics than among non-diabetics. Different causes of reduced resistance to infection have been discussed and inter alia the effect of the glucose concentration in the skin (Urbach 1945) The disposition to skin infections in connection with diabetes is considered to be due more to a poor general condition with malnutrition and dehydration as found in badly controlled diabetes (Marble 1959). A third group is generalized or localized pruritus which often appears in uncontrolled or poorly controlled diabetes. Finally changes may arise at the site of insulin injections, so-called lipodystrophy

Necrobiosis lipoidica diabeticorum consists of one or more distinctly demarcated, indurated bluish-red plaques with a smooth waxy surface generally located in the lower part of the leg. It usually begins as a papule that grows in size. In the later stages there is an extensive palmsized lesion with bluish-red, or brownish-red outer regions and a yellowish atrophic centre, at times with ulceration. As a rule it only gives cosmetic trouble. It occurs more often in young and middle aged women. This lesion of the skin was first observed by Oppenheim in 1929 but was named by Urbach in 1932. A similar change of the skin is also found among non-diabetics and Hare (1955) suggested the name necrobiosis lipoidica without the additional diabeticorum for such cases. In a study covering a period of twenty years from the Mayo Clinic Rollins and Winkelmann (1960) found 89 cases with necrobiosis lipoidica diabeticorum of which 29 were non-diabetics. Clinically the lesion in question appeared identical in diabetic and non-diabetic patients. Histologically however differences were found whereby it was suggested that the lesion in non-diabetics be named necrobiosis lipoidica granulomatosis.

The histopathological findings in necrobiosis lipoidica diabeticorum are areas of necrobiosis of the collagen throughout the dermis, especially in the lower parts. Within and near the areas of necrobiosis perivascular inflammatory infiltrates are found. The blood vessels in the dermis often exhibit fibrosis in the walls and proliferation in the endothelial lining occasionally with complete occlusion of

of the lower extremities in diabetes. The vascular lesions could be distinguished from arteriosclerotic lesions and were found in the vasa vasorum of the large vessels as well as in the small arteries of the nerves, muscles and skin. Similar findings are also reported by Ledersen and Olsen (1962). The symptom of intermittent claudication and objective signs of arterial insufficiency are also part of the picture of arterial disease in the lower extremities.

Similar diabetic vascular changes have also been reported concerning other vascular areas. Angervall et al. (1961) thus found in a resection and biopsy material from diabetic stomachs small vessel changes characterized by a deposit of PAS-positive material in the walls and endothelial proliferations.

As an expression of a more generalized capillary injury an increased capillary fragility is found in diabetics. It is often combined with retinopathy (Hanum 1939, Rodriguez and Root 1948). Circulation studies in diabetics with a short duration of the disease and without clinically proven diabetic vascular changes have revealed abnormal vascular reactions. Although interpretations have varied it is presumed that changes have occurred in the small vessels and that there had already been an incidence of angiopathy at an early stage (Megibow et al. 1953, Mendlowitz et al. 1953, Párány 1955, Sigroth 1957, Lax and Feinberg 1959, Aagenaes 1962).

Electron microscopic studies of particularly the capillary basement membrane in the renal glomeruli and in the retina as also in skin and muscle capillaries have revealed that the vascular changes may occur at an early stage in the course of the disease. Already in newly discovered diabetics of the maturity onset type and in young diabetics and even in prae-diabetics changes of the capillary basement membrane have been found with manifold thickening (Dayvog et al. 1961, Aagenaes and Moe 1961, Sabour et al. 1962, Zacks et al. 1962).

The question has arisen as to whether they are secondary to the diabetic metabolic disorder or if they are primarily hereditary changes.

CHAPTER II

DERMATOLOGIC LESIONS ASSOCIATED WITH DIABETES

There is a heterogeneous group of skin lesions that may occur in connection with diabetes. Although none of them is absolutely pathognomonic of diabetes they arise more often in diabetics than in non-diabetics. Among those skin changes generally considered to be diabetic» are necrobiosis lipoidica diabeticorum and xanthoma diabeticorum.

Another group consists of bacterial and mycotic skin infections. They are considered to be more common among diabetics than among non-diabetics. Different causes of reduced resistance to infection have been discussed and inter alia the effect of the glucose concentration in the skin (Urbach 1945). The disposition to skin infections in connection with diabetes is considered to be due more to poor general condition with malnutrition and dehydration as found in badly controlled diabetes (Marble 1959). A third group is generalized or localized pruritus which often appears in uncontrolled or poorly controlled diabetes. Finally changes may arise at the site of insulin injections, so-called lipodystrophy.

Necrobiosis lipoidica diabeticorum consists of one or more distinctly demarcated, indurated bluish-red plaques with a smooth waxy surface generally located in the lower part of the leg. It usually begins as a papule that grows in size. In the later stages there is an extensive palm-sized lesion with bluish-red, or brownish-red outer regions and a yellowish atrophic centre, at times with ulceration. As a rule it only gives cosmetic trouble. It occurs more often in young and middle aged women. This lesion of the skin was first observed by Oppenheim in 1929 but was named by Urbach in 1932. A similar change of the skin is also found among non-diabetics, and Hare (1955) suggested the name necrobiosis lipoidica without the additional diabeticorum for such cases. In a study covering a period of twenty years from the Mayo Clinic Rollins and Winkelmann (1960) found 89 cases with necrobiosis lipoidica diabeticorum of which 29 were non-diabetics. Clinically the lesion in question appeared identical in diabetic and non-diabetic patients. Histologically however differences were found whereby it was suggested that the lesion in non-diabetics be named necrobiosis lipoidica granulomatosis.

The histopathological findings in necrobiosis lipoidica diabeticorum are areas of necrobiosis of the collagen throughout the dermis, especially in the lower parts. Within and near the areas of necrobiosis perivascular inflammatory infiltrates are found. The blood vessels in the dermis often exhibit fibrosis in the walls and proliferation in the endothelial lining, occasionally with complete occlusion of

of the lower extremities in diabetics. The vascular lesions could be distinguished from arterio-arterioloscierosis and were found in the vasa vasorum of the large vessels as well as in the small arteries of the nerves, muscles and skin. Similar findings are also reported by Pedersen and Olsen (1962). The symptom of intermittent claudication and objective signs of arterial insufficiency are also part of the picture of arterial disease in the lower extremities.

Similar diabetic vascular changes have also been reported concerning other vascular areas. Angervall et al. (1961) thus found in a resection and biopsy material from diabetic stomachs, small vessel changes characterized by a deposit of PAS-positive material in the walls and endothelial proliferations.

As an expression of a more generalized capillary injury an increased capillary fragility is found in diabetics. It is often combined with retinopathy (Hanum 1939, Rodriguez and Root 1948). Circulation studies in diabetics with a short duration of the disease and without clinically proven diabetic vascular changes have revealed abnormal vascular reaction. Although interpretations have varied it is presumed that changes have ocurred in the small vessels and that there had already been an incidence of angiopathy at an early stage (Megibow et al. 1953, Mendlowitz et al. 1953, Bárány 1955, Sigroth 1957, Lax and Feinberg 1959, Aagenaes 1962).

Electron microscopic studies of particularly the capillary basement membrane in the renal glomeruli and in the retina, as also in skin and muscle capillaries, have revealed that the vascular changes may occur at an early stage in the course of the disease. Already in newly discovered diabetics of the maturity onset type, and in young diabetics and even in prae-diabetics changes of the capillary basement membrane have been found with manifold thickening (Daysog et al. 1961, Aagenaes and Moe 1961, Sabour et al. 1962, Zacks et al. 1962).

The question has arisen as to whether they are secondary to the diabetic metabolic discorder or if they are primarily hereditary changes.

CHAPTER III

ATROPHIC CIRCUMSCRIBED BROWNISH SKIN LESIONS IN THE LOWER EXTREMITIES OF DIABETICS

Since 1960 a study of the incidence of a circumscribed atrophic brownish skin lesion in the lower extremities of diabetics has been carried out at the Medical Clinic in Umeå. Gradually we have become convinced that the lesion is more or less specific for diabetes. We consider that we have gained a rather good conception of the appearance and localization of the lesion. With the aid of colour photography it has been possible to follow the lesions during the course of time and thus gain a conception of their development and duration. As far as we know these lesions have not previously been described in the literature. By studying the illustrated material in papers on diabetes we feel that we have discovered them in pictures from patients illustrating other changes (Foley and Wright 1959 Lundbaek 1960).

The lesions were almost exclusively located in the lower parts of the legs. They consisted of small light brown atrophic lesions either isolated or grouped. Sometimes they were arranged in a linear pattern or more irregularly in groups. The lesions varied in size, but they seldom exceeded 10 mm in diameter. In shape they were most often round or rounded, at times more extended. The atrophy appeared as a depression in the skin surface corresponding to the patch. The epidermis gave the impression of being thin and atrophic. The surface was often smooth and without crust. The atrophy varied from very distinct to hardly visible.

The lesions were brownish and clearly differed from the surrounding skin colour. The degree of pigmentation was correlated to the atrophy so that, as a rule, the most powerful pigmentation was found in the most pronounced atrophy. Where there was a less pronounced atrophy there was usually a lighter brown colour.

The lesions were localized in the front of the lower parts of the legs as well as on the medial and lateral sides. They were seldom found at the back of the legs. The lesions appeared on the bony parts of the legs as well as on the soft parts, and no definite distribution was found. The changes were often found on both legs, the one leg being sometimes more attacked than the other. The lesions were almost solely located in the lower parts of the legs and the feet were not affected. On single occasions the lesions have been found distal to the thigh. The lesions were not tender or itching. They are illustrated in Figs 6—14 at the end of the book.

As a result of repeated colour photography it has been possible to study the development of the lesions. It was found

the lumen Thrombosis in small vessels may even occur (Urbach 1932 Lever 1961)

The pathogenesis of necrobiosis lipoidica diabeticorum is not known Hare (1955) did not consider it to be the result of an inflammation or a local lipoidosis The significance of the changes in the vessels has also been discussed and the possibility of secondary infiltration an imbibition of blood lipoids has been suggested (Urbach 1932 Prud Homme 1960) External trauma has also been mentioned as an exacerbating factor in several reports (Fitzpatrick 1960)

Xanthoma diabeticorum is a rare lesion It is seen in patients with poorly controlled diabetes and is secondary to the hyperlipaemia The same lesions may also appear in other conditions with hyperlipemia The lesions often occur bilaterally as single or grouped papules and nodules of a yellowish colour on the buttocks, extensor surfaces palms and soles There may be marked pruritus with excoriations. The lesions disappear with the treatment of diabetes mellitus including proper restitution of carbohydrate utilization (Pillsbury et al 1957 Fitzpatrick 1960)

It is often said that diabetics have a disposition for skin infections It is, however considered that a properly treated diabetic has a normal resistance to skin infections (Fitzpatrick 1960)

The presence of mycotic infections of the vulva or in any area of the skin demands a search for diabetes mellitus and may occur in diabetics as eczematous changes in intertriginous regions (Pillsbury et al. 1957) The incidence of dermatophytoses is probably not higher in diabetics than in non-diabetics. However dermatophytosis of the interdigital spaces of the foot, causing epidermal fissures and erosions, may serve as a portal of entry for bacterial infections (Fitzpatrick 1960)

General pruritus may arise in diabetes. There is, however no connection between the itching and the severity of diabetes, and the former does not always vanish when the hyperglycemia and ketonuria are brought under control It has been suggested that the connection between the two conditions is merely coincidental (Guy 1951) Localized pruritus often occurs as pruritus vulvae in uncontrolled diabetes. If it does not vanish when the diabetes is under control an examination may usually disclose some local cause such as infection or some other gynaecological disorder

Lipodystrophy is a change in the subcutaneous fat at the site of insulin injections. It may develop a year or so after the commencement of insulin injections and may occur as a hypertrophy or an atrophy of the adipose tissue. It is relatively frequent particularly in women and children The pathogenesis is not clear but it is presumed that the action of insulin at the site of injection is metabolic and facilitates the synthesis of fat from carbohydrate. This should result in hypertrophy later followed by atrophy by a mechanism at present not known (Marble 1959)

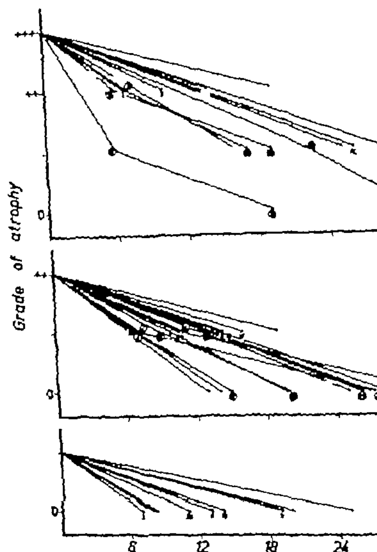


FIG 1 Duration j some selected atrophic skin lesion on the lower legs o
betes studied by colour photography. Atrophy is graded in rough scale
patients are numbered from 1 to 20. The encircled figur denote lesion
tumes.

1 B M 21 years fem. Diab 15 year — *11 P R. 68 years male.*
2 S.L. 40 male Diab 20 — *12 R.M 59 male*

that the atrophy and pigmentation diminished almost parallelly and very slowly On an average from one and a half to two years later the lesions had disappeared and only an insignificant atrophy distinguished them from their surroundings. The brownish colour had then also vanished and sometimes been replaced by a slight depigmentation Sometimes the lesions had completely vanished In the meanwhile new lesions might have arisen The total picture therefore appeared unchanged as a rule. In reality the single skin lesion continuously changed towards a restitution Fig 1 schematically shows duration and restitution in some atrophic skin lesions observed in a series of technicolour pictures from 20 patients.

The patients were not often conscious of the existence of the lesions Only a few patients made remarks concerning the type of occurrence. The majority of the patients whose attention was called to the changes presumed that they were traumatic without being able to determine any particular trauma More often than not it went far back in time, which lessened the certainty of the statement. On the other hand some patients stated that they were subject to spontaneously occurring sores on their legs that took a long time to heal Others again asserted that they easily got sores on their legs and considered them to have been caused by insignificant injuries

Despite an intensive search it was difficult to determine the appearance of the primary lesion Sometimes small superficial ulcers were found but they were probably several weeks old and statements regarding their occurrence were as diffuse as they were varied. On one occasion we observed what we thought to be the primary lesion It concerned a patient who on examination was seen to have atrophic skin lesions of the described type and who two days after the examination stated that he had spontaneously got a new rash on the legs There were some intensively red macules of about the same size as the observed atrophic skin lesions and which did not disappear on pressure (Fig 6a) Later they developed into typical atrophic lesions (Fig 6b) In this case the type of incidence was spontaneous.

On some patients traumatization was performed with a hard rubber hammer but no changes arose. The question of the incidence type must yet remain unanswered

The differential diagnosis of the atrophic skin lesions involve some other atrophic conditions of the skin Papulonecrotic and aceniform tuberculids heal with the formation of circular sharply demarcated small scars, which are atrophic. They are often pale often with a slight pigmented border zone. They usually occur on the extremities and in the early stages may reveal histologically a tuberculoid structure (cf Sutton 1956 Pillsbury et al 1957) The atrophic skin lesions in diabetics were only found on the lower extremities and papulonecrotic lesions were not observed. The atrophic lesions in diabetics disappear in contrast to the scars after healed tuberculids, and the histopathological picture of the lesions is not in agreement with that often seen in tuberculids (see page 43) Neurotic excoriations may appear as pigmented and sometimes slightly atrophic

CHAPTER IV

THE PATIENT MATERIAL

The material consisted of 293 patients with diabetes. A non-diabetic material used for comparison consisted of 104 patients with hypertension. All of them were admitted to the Medical Clinic in Umeå. All the patients with diagnosed diabetes who had received treatment during the period 1960—1962 were examined, and the comparative material was investigated consecutively during the same period (Melm 1962). A great number of the diabetic patients with previously described skin lesions were examined several times in order to study the course of the skin changes.

DIAGNOSTIC CRITERIA

All the diabetic patients were examined for so-called diabetic complications. With reference to retinopathy practically all the patients, and certainly all the doubtful cases, were examined by an ophthalmologist. The latter examined about 90 per cent of the cases, and the rest was examined by myself.

Retinopathy denotes the incidence of microaneurysms only or in association with haemorrhages, exudates and vascular proliferations. Patients with media opacities that prevented the ophthalmological examination were registered as cases without retinopathy. If the retinopathy was established previous to the incidence of the media-opacities, the cases were however registered as retinopathy. Patients with advanced media-opacities, where the eye-ground could not be satisfactorily inspected, formed a relatively small part of the material — only 6 cases.

Nephropathy was registered if on repeated occasions, albuminuria arose without simultaneous signs of urinary tract infection. In those cases where there was a combination of albuminuria and urinary tract infection, the degree of albuminuria proved decisive for registration. Patients with a massive and constant albuminuria were registered as nephropathy. In those cases where there were also other albuminuric causal factors, such as cardiac decompensation or malignant hypertension, the diagnosis of diabetic nephropathy was, owing to natural reasons, uncertain. Doubtful cases have not been registered as nephropathy.

The frequence of neuropathy is partly dependent upon the criteria used for the diagnosis. The occurrence of subjective symptoms has not been dominant in the material. The dominant objective finding was a bilateral lack of achilles reflexes, and sometimes of the patella reflexes, too. A reduced, or lost vibration sense over the malleoli was also often observed. The vibration sense was tested with a tuning fork (C 64). Neuropathy was

lesions on any part of the body (Sutton 1956) They are often more irregular in shape than the lesions in diabetics and are an expression of a psychopathologic condition. In connection with chronic venous insufficiency in the lower extremities pigmented slight atrophic lesions are sometimes seen (Dodd and Cockett 1956) They are often isolated and are secondary to the varicose disease.

Other dermatologic diseases such as e. g lichen planus often leave pigmented scars after healing The case history and the distribution of the scars usually render the diagnosis of the primary disease clear

Joslin et al 1959). The youngest patient was a 15 year old boy and the eldest a woman of 86 years of age. The median age for the men was 50.0 years, and for the women 60.2 years. The median age in the whole material was 55.3 years.

Of 130 men 78 had previously described atrophic skin lesions on the legs, whereas 10 had somewhat divergent skin changes. Of 163 women 46 hade atrophic skin lesions on the lower legs, whereas 6 had somewhat divergent skin changes. In the continued studies of frequency the two groups with divergent skin changes were not included. The material consisted then of 120 men 78 of whom had atrophic skin lesions (65 per cent) and 157 women of whom 46 had atrophic skin lesions (29 per cent) The groups with divergent skin changes will later be dealt with separately

The frequence of atrophic skin lesions in men and women was practically the same in the different age groups (Table 1) The youngest age group was an ex-

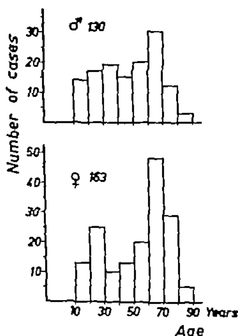


FIG 2 *Age distribution of diabetic men and women.*

TABLE 1 *The frequence of atrophic skin lesions in different age groups in diabetic men and women*

Age	Men Cases	Per cent	Age	Women Cases	Per cent
10—19	2/13	15	10—19	1/13	8
20—29	12/16	75	20—29	8/23	35
30—39	12/18	67	30—39	5/9	56
40—49	10/14	71	40—49	3/12	25
50—59	11/18	61	50—59	5/18	28
60—69	20/28	71	60—69	18/48	38
70—7	10/11	91	70—79	5/29	17
80—89	1/2	50	80—89	1/5	20
Total	78/120	65	Total	46/157	29

registered where there was a bilateral lack of achilles reflexes with, or without a combination of weakened or extinct patella reflexes and reduced or lost sense of vibration. A prerequisite for only a double sided achilles reflex being registered as neuropathy was that no other reason for such areflexia existed and that the patient was without signs of ketonuria Doubtful cases were not registered as neuropathy

Hypertension was registered for diastolic pressure exceeding 100 mm Hg The blood pressure was measured on several occasions and always during rest In patients under 40 years of age a diastolic blood pressure exceeding 90 mm Hg was registered as hypertension

Arterial disease in the lower extremities was assessed with the guidance of subjective symptoms in the form of intermittent claudication and objective findings such as the absence of palpable foot pulses and pallor of the foot when the leg was elevated The incidence of gangrene or healed gangrene in the feet was similarly noted. That which was decisive for the diagnosis was subjective symptoms of intermittent claudication or the incidence of gangrene or the absence of palpable foot pulses in association with plantar blanching

The atrophic skin lesions on the lower legs were registered with colour photography (Contarex, Zeiss-Ikon with Planar 1 2 f = 50 mm. and Sonar 1 2 f = 85 mm Film Kodachrome Type A Two Philips 500 watt lamps) The legs were partly photographed in toto and in part the skin changes were photographed at close range. The colour slides especially in doubtful cases, have been of great help in the final analysis of the lesions.

The comparative material of patients with hypertension and without diabetes was examined in the same manner The occurrence of atrophic skin changes on the legs corresponding to that observed in diabetics was registered. The incidence of hypertensive vascular changes in the retina according to Keith-Wagener was, in the majority of cases, assessed by an ophthalmologist. As malignant hypertension cases were registered with eye-ground changes of the 3rd and 4th degree and a diastolic blood pressure exceeding 130 mm Hg Otherwise the examination was carried out in the same way as for the diabetic patients. The statistic significance was assessed according to the Chi Square method (= χ^2) Degrees of freedom (*df*) and significance limits for P are given Yates's correction was used when samples were small (Guilford 1950)

THE DIABETIC MATERIAL

The diabetic material consisted of 130 men and 163 women The distribution of the material in relation to sex and age groups in seen in Fig 2 Among th women especially a double peak distribution was found with a maximum frequence in the group 20 to 29 years of age and one in the age group 60 to 69 The relation between men and women was about 1 1 and similarly in the different age groups with the exception of those age groups exceeding 60 years where the women dominated. A similar distribution has been demonstrated in other materials (Dahlberg et al 1947

Joslin et al 1959). The youngest patient was a 15 year old boy and the eldest a woman of 86 years of age. The median age for the men was 50.0 years, and for the women 60.2 years. The median age in the whole material was 55.3 years.

Of 130 men 78 had previously described atrophic skin lesions on the legs, whereas 10 had somewhat divergent skin changes. Of 163 women 46 hade atrophic skin lesions on the lower legs, whereas 6 had somewhat divergent skin changes. In the continued studies of frequency the two groups with divergent skin changes were not included. The material consisted then of 120 men 78 of whom had atrophic skin lesions (65 per cent), and 157 women of whom 46 had atrophic skin lesions (29 per cent) The groups with divergent skin changes will later be dealt with separately

The frequence of atrophic skin lesions in men and women was practically the same in the different age groups (Table 1) The youngest age group was an ex

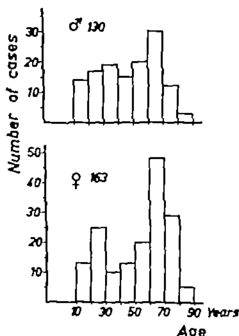


FIG. 2. *Age Distribution of diabetic men and women.*

TABLE 1 *The frequence of atrophic skin lesions in different age groups in diabetic men and women.*

Age	Men Cases	Men Per cent	Age	Women Cases	Women Per cent
10—19	2/13	15	10—19	1/13	8
20—29	12/16	75	20—29	8/23	35
30—39	12/18	67	30—39	5/9	56
40—49	10/14	71	40—49	3/12	25
50—59	11/18	61	50—59	5/18	28
60—69	20/28	71	60—69	18/48	38
70—79	10/11	91	70—79	5/29	17
80—89	1/2	50	80—89	1/5	20
Total	78/120	65	Total	46/157	29

registered where there was a bilateral lack of achilles reflexes with or without a combination of weakened, or extinct patella reflexes and reduced or lost sense of vibration. A prerequisite for only a double sided achilles reflex being registered as neuropathy was that no other reason for such areflexia existed and that the patient was without signs of ketonuria. Doubtful cases were not registered as neuropathy

Hypertension was registered for diastolic pressure exceeding 100 mm. Hg The blood pressure was measured on several occasions and always during rest In patients under 40 years of age a diastolic blood pressure exceeding 90 mm Hg was registered as *hypertension*

Arterial disease in the lower extremities was assessed with the guidance of subjective symptoms in the form of intermittent claudication and objective findings such as the absence of palpable foot pulses and pallor of the foot when the leg was elevated The incidence of gangrene or healed gangrene in the feet was similarly noted. That which was decisive for the diagnosis was subjective symptoms of intermittent claudication or the incidence of gangrene or the absence of palpable foot pulses in association with plantar blanching

The atrophic skin lesions on the lower legs were registered with colour photography (Contarex Zeiss-Ikon with Planar 1 2 f = 50 mm and Sonar 1 2 f = 85 mm Film Kodachrome Type A Two Philips 500 watt lamps) The legs were partly photographed in toto, and in part the skin changes were photographed at close range. The colour slides, especially in doubtful cases, have been of great help in the final analysis of the lesions

The *comparative material* of patients with hypertension and without diabetes was examined in the same manner The *occurrence* of atrophic skin changes on the legs corresponding to that observed in diabetics was registered. The *incidence of hypertensive vascular changes* in the retina according to Keith-Wagener was in the majority of cases *assessed by an ophthalmologist* As malignant hypertension cases were registered with eye-ground changes of the 3rd and 4th degree and a diastolic blood pressure exceeding 130 mm Hg Otherwise the examination was carried out in the same way as for the diabetic patients. The statistic significance was assessed according to the Chi Square method (= χ^2) Degrees of freedom (df) and significance limits for P are given. Yates's correction was used when samples were small (Guilford 1950)

THE DIABETIC MATERIAL

The diabetic material consisted of 130 men and 163 women The distribution of the material in relation to sex and age groups in seen in Fig 2 Among th women especially a double peak distribution was found with a maximum frequence in the group 20 to 29 years of age and one in the age group 60 to 69 The relation between men and women was about 1 1 and similarly in the different age groups with the exception of those age groups exceeding 60 years where the women dominated. A similar distribution has been demonstrated in other materials (Dahlberg et al 1947

than 10 years (Men $\chi^2 = 23.4$ df = 1 $P < 0.001$ women χ^2 15.0, df = 1 $P < 0.001$)

The frequence of atrophic skin lesions in relation to age and diabetic duration is seen in Table 3 It will be seen that the atrophic skin changes in the oldest age group occurred after a short diabetic duration in comparison with the youngest age group where they occurred only after a 10 year diabetic duration. Thus in the younger age groups there was a longer diabetic duration before the skin changes arose compared to the older groups. An increase of the atrophic skin lesions was, however found in all the age groups with increasing diabetic duration.

In the oldest age group with a diabetic duration less than 5 years there were 22 patients with and 37 without atrophic skin lesions. Of the patients with atrophic skin changes 12 were men and 10 women. Of the 22 patients mentioned with atrophic skin changes 6 had retinopathy and in 3 there were media-opacities which made an examination of the retinal vessels impossible. None of the 37 patients without skin lesions had retinopathy Among the latter however there were two patients with bilateral cataracts which prevented inspection. Judging from the frequence of retinopathy it would appear as if the patients with atrophic skin changes should have had a longer diabetes-duration than five years when compared with patients without these changes. It is well known that the onset of diabetes is difficult to determine in old age.

Retinopathy

It is generally agreed that diabetic retinopathy is correlated to the duration of diabetes. As to whether diabetic retinopathy appears after a shorter duration of diabetes in elderly than in young diabetics is not quite clear Dolger (1947) found that retinopathy occurs somewhat earlier in elderly diabetics in comparison to younger ones. He had, however no patients over 50 years of age in his material. Pyke and Roberts (1959) found retinopathy in 6.4 per cent of elderly patients in connection with the discovery of their diabetes.

TABLE 3 *The frequence of atrophic skin lesions in diabetics in relation to age and diabetic duration*

Duration Years	Age groups < 20	%	20—39	%	40—59	%	> 60	%	Total	%
0— 4	0/11	—	0/10	—	5/26	19	22/59	37	27/106	25
5— 9	0/9	—	3/11	27	7/12	58	9/24	38	19/56	34
10—14	3/6	50	7/14	50	6/11	55	17/26	65	33/57	58
>15	—	—	27/31	90	11/13	85	7/14	50	45/58	78
Total	3/26	12	37/66	56	29/62	43	51/123	41	124/277	45

ception in that the frequence of skin changes was low for both men and women In the oldest age groups the number of patients was small and thus the frequence figures were uncertain There was no significant difference in frequence below and above the median age for men and women (Men $\chi^2 = 0.916$ df = 1 0.4> P> 0.3 Women $\chi^2 = 0.279$ df = 1 0.6> P> 0.5 Yates's correction)

If the frequence of skin lesions was calculated in relation to the duration of the diabetic disease instead it was seen *that in men as well as in women the frequence* of atrophic skin changes increased with an increasing diabetic duration (Table 2) The men had percentually a higher frequence of atrophic skin changes in the lower duration groups compared to the women The frequence of atrophic skin changes was highest in the groups with a diabetic duration exceeding 15 years both for men and women the difference in frequence between the sexes being less marked The number of *patients in the longest duration* groups was, however, small and therefore the frequence figures uncertain Of the patients who had had diabetes for more than 30 years were 6 men one of whom lacked skin lesions. He was a 66 year old man and had had diabetes for 31 years With the exception of the last few years, when he was treated with sulphonylureas, he had been subject only to dietetic treatment He showed no signs of diabetic complications In the same duration group were 3 women 2 of whom lacked skin changes. The one was 36 years old with a diabetic duration of 30 years and with only insignificant retinopathy The other was 61 years old with a diabetic duration of 32 years and she, too had only an insignificant retinopathy and a slight neuropathy

The frequence of atrophic skin lesions after more than 10 years diabetic duration was significantly higher than in patients with a diabetic duration of less

TABLE 2. *The frequence of atrophic skin lesions in relation to diabetic duration in men and women.*

Duration Years	Men Cases	Per cent	Duration Years	Women Cases	Per cent
0— 4	17/43	40	0— 4	10/63	16
5— 9	10/18	56	5— 9	9/38	24
10—14	22/27	82	10—14	11/30	37
15—19	16/18	89	15—19	7/11	64
20—24	5/5	100	20—24	6/10	60
25—29	3/3	100	25—29	2/2	100
> 30	5/6	83	> 30	1/3	33
Total	78/120	65	Total	46/157	29

than 10 years (Men $\chi^2 = 23.4$ df $= 1$ $P < 0.001$ women $\chi^2 = 15.0$ df $= 1$ $P < 0.001$)

The frequence of atrophic skin lesions in relation to age and diabetic duration is seen in Table 3. It will be seen that the atrophic skin changes in the oldest age group occurred after a short diabetic duration in comparison with the youngest age group where they occurred only after a 10 year diabetic duration. Thus in the younger age groups there was a longer diabetic duration before the skin changes arose compared to the older groups. An increase of the atrophic skin lesions was, however, found in all the age groups with increasing diabetic duration.

In the oldest age group with a diabetic duration less than 5 years there were 22 patients with and 37 without atrophic skin lesions. Of the patients with atrophic skin changes 12 were men and 10 women. Of the 22 patients mentioned with atrophic skin changes 6 had retinopathy and in 3 there were media-opacities which made an examination of the retinal vessels impossible. None of the 37 patients without skin lesions had retinopathy. Among the latter however there were two patients with bilateral cataracts which prevented inspection. Judging from the frequence of retinopathy it would appear as if the patients with atrophic skin changes should have had a longer diabetes-duration than five years when compared with patients without these changes. It is well known that the onset of diabetes is difficult to determine in old age.

Retinopathy

It is generally agreed that diabetic retinopathy is correlated to the duration of diabetes. As to whether diabetic retinopathy appears after a shorter duration of diabetes in elderly than in young diabetics is not quite clear. Dolger (1947) found that retinopathy occurs somewhat earlier in elderly diabetics in comparison to younger ones. He had, however, no patients over 50 years of age in his material. Pyke and Roberts (1959) found retinopathy in 6.4 per cent of elderly patients in connection with the discovery of their diabetes.

TABLE 3. *The frequence of atrophic skin lesions in diabetics in relation to age and diabetic duration*

Duration Years	< 20	%	20—39	%	Age groups 40—59	%	> 60	%	Total	%
0— 4	0/11	—	0/10	—	5/26	19	22/59	37	27/106	25
5— 9	0/9	—	3/11	27	7/12	58	9/24	38	19/56	34
10—14	3/6	50	7/14	50	6/11	55	17/26	65	33/57	58
>15	—	—	27/31	90	11/13	85	7/14	50	45/58	78
Total	3/26	12	37/66	56	29/62	43	55/123	41	124/277	45

The frequence of retinopathy in relation to age and diabetic duration in this material of diabetic patients is seen in Table 4 It will be seen also here that the diabetic duration previous to the onset of retinopathy decreased with increasing age. In the youngest age group retinopathy only arose after a 10 year diabetic duration whereas in the oldest age group it was found in 10 per cent in the group with less than 5 years diabetic duration In the total column to the right of the Table it will be seen also how the frequence of retinopathy increased with increasing diabetic duration After more than 15 years of diabetic duration so-called long term diabetes, the frequence of retinopathy was 90 per cent. This figure is rather high in comparison with other materials (Dolger 1947 Mårtensson 1950 Lundbaek 1953). Lundbaek found in a long-term material from Aarhus in Denmark a frequence of retinopathy of 80 per cent, while Mårtensson in a similar Swedish material only found a frequence of 40 per cent. The highest frequence 100 per cent is reported from the U S A. by Dolger on diabetics with a duration of diabetes of up to twenty five years.

The frequence of retinopathy in men and women with and without atrophic skin lesions respectively is seen in Table 5 There was a total of 86 patients (69 per cent) with retinopathy in the group with atrophic skin changes as compared

TABLE 4 *The frequence of retinopathy in diabetics in relation to age and diabetic duration.*

Duration Years	Age groups < 20	%	20—39	%	40—59	%	> 60	%	Total	%
0— 4	0/11	—	0/10	—	2/26	8	6/59	10	8/106	8
5— 9	0/9	—	5/11	46	7/12	58	9/24	38	21/56	38
10—14	5/6	83	12/14	86	8/11	73	18/26	69	43/57	75
>15	—	—	31/31	100	11/13	85	10/14	71	5[illegible]/58	90
Total	5/26	19	48/66	73	28/62	45	43/123	35	124/277	45

TABLE 5 *The frequence of retinopathy in diabetic men and women with and without atrophic skin lesions*

	With Cases	%	Without Cases	%	Total Cases	%
Men	54/78	69	6/42	14	60/120	50
Women	32/46	70	32/111	29	64/157	41
Total	86/124	69	38/153	25	1 4/277	45

with 38 patients (25 per cent) in the group without such lesions. The difference in frequence of retinopathy between the groups with and without atrophic skin changes was significant for both men and women (Men χ^2 = 33.0 df = 1 P < 0.001 Women χ^2 = 22.4 df = 1 P < 0.001).

Exudative and proliferative retinopathy was found in 39 per cent (48 patients) in the group with atrophic skin lesions as against only 9.8 per cent (15 patients) in the group without such lesions. The ratio of the total frequence of retinopathy in the group with and without skin changes was 69 25 or 2.8 1 The corresponding ratio for the advanced retinopathy was 39 9.8 or 4 1 Proportionately there were thus more patients with advanced retinopathy in the group with atrophic skin changes than in the one without. The difference was probably significant (χ^2 3.30 df 1 0.1 > P > 0.05 Yates's correction).

Diabetic nephropathy

Diabetic nephropathy is, like retinopathy correlated to the diabetic duration and is considered to occur with about the same frequence in men women. It has sometimes been rep that there is a predominance among men in the younger age groups 1953)

There were 41 patients (15 per with nephropathy in this material frequence was about the same for and women, 17 and 13 per cent r tively (Table 6) The frequence of ropathy in the group with atroph lesions was significantly greater th the group without. (χ^2 24.8 df P < 0.001).

The frequence of nephropathy in tion to age and diabetic duration in Table 7 The same tendency w served here as in diabetic retino Previous to the onset of nephr diabetic duration decreased with sing age. In the oldest age group was a patient with nephropathy of the lowest duration groups, w in the youngest age group there 2 patients with nephropathy only a 10 year diabetic duration The n of cases in the different durati groups was small and permitted t mite assessment of the frequence

TABLE 6 *The frequence of nephropathy in diabetic men and women with and without at skin lesions.*

The frequence of retinopathy in relation to age and diabetic duration in this material of diabetic patients is seen in Table 4. It will be seen also here that the diabetic duration previous to the onset of retinopathy decreased with increasing age. In the youngest age group retinopathy only arose after a 10 year diabetic duration, whereas in the oldest age group it was found in 10 per cent in the group with less than 5 years diabetic duration. In the total column to the right of the Table it will be seen also how the frequence of retinopathy increased with increasing diabetic duration. After more than 15 years of diabetic duration, so-called long term diabetes, the frequence of retinopathy was 90 per cent. This figure is rather high in comparison with other materials (Dolger 1947, Mårtensson 1950, Lundbaek 1953). Lundbaek found in a long-term material from Aarhus in Denmark a frequence of retinopathy of 80 per cent, while Mårtensson in a similar Swedish material only found a frequence of 40 per cent. The highest frequence, 100 per cent, is reported from the U. S. A. by Dolger on diabetics with a duration of diabetes of up to twenty-five years.

The frequence of retinopathy in men and women with and without atrophic skin lesions respectively is seen in Table 5. There was a total of 86 patients (69 per cent) with retinopathy in the group with atrophic skin changes as compared

TABLE 4. *The frequence of retinopathy in diabetics in relation to age and diabetic duration*

Duration Years	Age groups < 20	%	20—39	%	40—59	%	> 60	%	Total	%
0— 4	0/11	—	0/10	—	2/26	8	6/59	10	8/106	8
5— 9	0/9	—	5/11	46	7/12	58	9/24	38	21/56	38
10—14	5/6	83	12/14	86	8/11	73	18/26	69	43/57	75
> 15	—	—	31/31	100	11/13	85	10/14	71	52/58	90
Total	5/26	19	48/66	73	28/62	45	43/123	35	124/277	45

TABLE 5. *The frequence of retinopathy in diabetic men and women with and without atrophic skin lesions*

	With Cases	%	Without Cases	%	Total Cases	%
Men	54/78	69	6/42	14	60/120	50
Women	32/46	70	32/111	29	64/157	41
Total	86/124	69	38/153	25	124/277	45

TABLE 8 *The frequence of neuropathy in diabetic men and women with and without atrophic skin lesions.*

	With		Without		Total	
	Cases	%	Cases	%	Cases	%
Men	47/78	60	5/42	12	52/120	43
Women	23/46	50	25/111	23	48/157	31
Total	70/124	57	30/153	20	100/277	36

men x^2 10 3 df 1 $0.005 > P > 0.001$ Yates's correction). The frequence of neuropathy for the whole material was 36 per cent, for the men 43 per cent, for the women 31 per cent. The men had a somewhat higher frequence of neuropathy than the women. (x^2 = 4 80. df 1 $0.05 > P > 0.03$) As previously mentioned the frequence figures vary for different materials, but largely they range from about 40 to 65 per cent (cf Steiness 1963) In two investigations of diabetic neuropathy carried out by Fagerberg (1959) and Steiness (1963) the corresponding figures were 63 and 52 per cent respectively In a long-term material with more than 15 years duration Lundbaek (1953) found only 32 per cent with achilles areflexia. If weak achilles reflexes also were included the figure rose to 45 per cent.

The frequence of neuropathy in relation to age and diabetic duration is given in Table 9 As is seen in the lower row in the Table the total frequence of neuropathy was low in the youngest age group in comparison with the remainder The number of patients in this group was, however small, and there were comparatively few cases with a diabetic duration exceeding 10 years. In the other age groups the frequence of neuropathy was about as great. It has been stated that the frequence of diabetic neuropathy is greater in patients over 60 years of age than in those below this age limit (Jordan 1936 Fagerberg 1959 Steiness 1963) No difference in this respect is found in this material (x^2 = 0.023 df = 1 $0.9 > P > 0.8$)

In all the age groups the frequence of neuropathy increased with increasing diabetic duration. The total frequence of neuropathy in the different duration groups is given in the right column of the Table. The frequence rose evenly with increasing diabetic duration except in the group with the longest duration. The frequence in this group increased only inconsiderably compared to the duration group 10 to 14 years. Steiness (1963) found in Lundbaeks material a similar condition in the groups with 15 to 19 and 20 to 25 years duration. Discussions have taken place as to whether the frequence of diabetic neuropathy becomes constant after a certain diabetic duration, or if some selective factor is in action (Steiness 1963)

Earlier in the presentation a division was made of the frequence of retino- and nephropathy in relation to age and

TABLE 7 *The frequence of nephropathy in diabetics in relation to ge and diabetic duration*

Duration Years	Age groups: < 20	%	20—39	%	40—59	%	> 60	%	Total	%
0—4	0/11	—	0/10	—	0/26	—	1/59	2	1/106	1
5—9	0/9	—	0/11	—	1/1	8	1/24	4	2/56	4
10—14	2/6	33	5/14	36	1/11	9	6/26	23	14/57	25
<15	—	—	19/31	61	3/13	23	2/14	14	24/58	40
Total	2/26	8	24/66	39	5/62	8	10/123	8	41/277	15

total frequence in the different duration groups is seen in the right column of the Table. It increased evenly with increasing diabetic duration. Of the 58 patients with a diabetic duration exceeding 15 years — long-term diabetes — 24 (41 per cent) had nephropathy In long term diabetic materials studied by Mårtensson (1950) and Lundback (1953) the corresponding figures were 33 and 58 per cent respectively

The frequence of nephropathy in this material in the different age groups appeared to be higher in the two lowest and if the material was divided into two groups, under and over 40 years, the frequence of nephropathy was significantly higher in the younger than in the older group (χ^2 = 18 3 df = 1 P< 0 001 Yates s correction) This may be partly due to the fact that nephropathy in the older age groups may often be blurred by urinary tract infection hence the clinical picture being more difficult to assess. Moreover percentually there were more patients with long-term diabetic duration in the younger group than in the older one.

The fact that nephropathy gives rise to a poor prognosis was seen in that 15 of the 41 cases with nephropathy died during the period of examination Post mortem examinations were performed on 9 of these patients and in 7 of them glomerulosclerotic changes were established according to Kimmelstiel-Wilson The remainder had nephrosclerosis without signs of glomerulosclerosis. With the exception of one all the patients had previous to death signs of increasing renal insufficiency with uraemia.

Neuropathy

More and more support has been given to the opinion that diabetic neuropathy has a vascular origin It has been proved that it is correlated to diabetic duration and to other so-called diabetic complications, above all retinopathy and nephropathy (cf Fagerberg 1959)

The frequence of neuropathy in patients with and without atrophic skin lesions is seen in Table 8 There is a considerably greater frequence of neuropathy in patients with atrophic skin changes, men as well as women compared with patients without such changes. The difference is significant for both men and women. (Men χ^2 = 24 1 df = 1 P< 0,001 Yates s correction Wo-

TABLE 8 *The frequence of neuropathy in diabetic men and women with and without atrophic skin lesions.*

	With		Without		Total	
	Cases	%	Cases	%	Cases	%
Men	47/78	60	5/42	12	52/120	43
Women	23/46	50	25/111	23	48/157	31
Total	70/124	57	30/153	20	100/277	36

men (χ^2 = 10.3 df = 1 $0.005 > P > 0.001$ Yates's correction). The frequence of neuropathy for the whole material was 36 per cent, for the men 43 per cent, for the women 31 per cent. The men had a somewhat higher frequence of neuropathy than the women (χ^2 = 4.80 df = 1 $0.05 > P > 0.03$). As previously mentioned the frequence figures vary for different materials, but largely they range from about 40 to 65 per cent (cf. Steiness 1963). In two investigations of diabetic neuropathy carried out by Fagerberg (1959) and Steiness (1963) the corresponding figures were 63 and 52 per cent respectively. In a long-term material with more than 15 years' duration Lundbaek (1953) found only 32 per cent with achilles areflexia. If weak achilles reflexes also were included the figure rose to 43 per cent.

The frequence of neuropathy in relation to age and diabetic duration is given in Table 9. As is seen in the lower row in the Table the total frequence of neuropathy was low in the youngest age group in comparison with the remainder. The number of patients in this group was, however, small, and there were comparatively few cases with a diabetic duration exceeding 10 years. In the other age groups the frequence of neuropathy was about as great. It has been stated that the frequence of diabetic neuropathy is greater in patients over 60 years of age than in those below this age limit (Jordan 1936, Fagerberg 1959, Steiness 1963). No difference in this respect is found in this material. (χ^2 = 0.023 df = 1 $0.9 > P > 0.8$)

In all the age groups the frequence of neuropathy increased with increasing diabetic duration. The total frequence of neuropathy in the different duration groups is given in the right column of the Table. The frequence rose evenly with increasing diabetic duration except in the group with the longest duration. The frequence in this group increased only inconsiderably compared to the duration group 10 to 14 years. Steiness (1963) found in Lundbaek's material a similar condition in the groups with 15 to 19 and 20 to 25 years' duration. Discussions have taken place as to whether the frequence of diabetic neuropathy becomes constant after a certain diabetic duration, or if some selective factor is in action (Steiness 1963).

Earlier in the presentation a division was made of the frequence of retino- and nephropathy in relation to age and

TABLE 9 *The frequence of neuropathy in diabetics in relation to age and diabetic duration*

Duration Years	Age groups < 20	%	20—39	%	40—59	%	> 60	%	Total	%
0— 4	0/11	—	0/10	—	3/26	12	15/59	25	18/106	17
5— 9	2/9	22	1/11	9	5/12	42	8/24	33	16/56	29
10—14	3/6	50	9/14	64	5/11	46	15/26	58	32/57	56
> 15	—	—	17/31	55	10/13	77	7/14	50	34/58	59
Total	5/26	19	27/66	41	23/62	37	45/123	37	100/277	36

diabetic duration whereby it was seen that diabetic duration previous to the onset of those complications was longest in the youngest age group but subsequently bated with increasing age and that in the oldest age group there was already a number of cases with complications in connection with the onset of diabetes A corresponding condition was found for neuropathy but not so pronounced. In the youngest age group neuropathy arose already after a 5 year diabetic duration and in the oldest age group there were 15 patients with neuropathy prior to a 5 year diabetic duration The number af cases was however small and did not permit a closer interpretation As a possible alternative to diabetic neuropathy occurring earlier than other so-called complications it is possible that the diagnostic criteria have not been adequate or that apart from the vascular origin there may be a contributory nonduration-conditioned factor Neuropathy can lead to the discovery of diabetic disease in higher ages, or it may occur early after the onset of diabetes. (Rundles 1945 Martin 1953) In the oldest group with less than 5 years diabetic duration neuropathy was found in one fourth of the patients

In the majority of cases diabetic neuropathy gave little or no subjective symptoms and where such existed it was often difficult to decide if it was due or not to neuropathy In 13 (10 man and 3 women) of the 100 cases with neuropathy this was more pronounced with severe subjective symptoms often with uncertainty in the gait and fear of faulty stepping The objective findings in this group were also more pronounced with lost achilles and patella reflexes lost sense of vibration impaired sensibility and sometimes pareses. These cases were classified severe. They occurred in all age groups with the exception of the youngest and appeared to be somewhat more common below than above 60 years of age (9 and 4 cases respectively) Seven of the 13 cases with severe neuropathy had a diabetic duration exceeding 15 years the remainder being evenly distributed in the lower duration groups. With the exception of one all the patients had atrophic skin lesions on the legs. One might suppose that in this group neuropathy was of significance as regards the

occurrence of the skin changes, particularly by way of a traumatic origin. The group was, however small and the severe neuropathy could therefore hardly be of any decisive importance to the incidence of the atrophic skin lesions on the whole.

Arterial disease in the lower extremities

In the material there were 38 patients (14 per cent) with arterial disease in the lower extremities either in the form of gangrene in the feet, or in the form of intermittent claudication with obvious signs of arterial insufficiency

Table 10 shows the frequence of arterial disease in the lower extremities in men and women with and without atrophic skin lesions. There was a total of 20 per cent in the group with atrophic skin changes compared with 9 per cent in the group without such changes. The difference was significant (χ^2 6.92 df – 1 0.01 > P > 0.005 Yates correction). In the group without atrophic skin changes the frequence difference of arterial disease was great between men and women — 0 and 12 per cent respectively The difference was due to the men in this group being considerably younger than the women. The frequence of arterial disease of the lower extremities in the whole material was 14 per cent and here there was no difference between men and women. This similarity in the frequence of peripheral arterial disease

TABLE 10 *The frequence of arterial disease in the lower extremities in diabetic men and women with and without atrophic skin lesions*

	With		Without		Total	
	Cases	%	Cases	%	Cases	%
Men	17/78	22	0/42	—	17/120	14
Women	8/46	17	13/111	12	21/157	13
Total	25/124	20	13/153	9	38/277	14

TABLE 11 *The frequence of peripheral arterial disease in the lower extremities in diabetics in relation to age and diabetic duration.*

Duration	Age groups					
Years	<60	%	>60	%	Total	%
0— 4	4/47	9	10/59	17	14/106	13
5— 9	3/32	9	2/24	8	5/56	9
10—14	2/31	6	10/26	38	12/57	21
>15	5/44	11	2/14	14	7/58	12
Total	14/154	9	24/123	20	38/277	14

in diabetic men and women has often been put forward and differs considerably from corresponding conditions in non-diabetic men and women where the former are extremely dominant (Dry and Hines 1941 Bell 1957)

The frequence of arterial disease in the lower extremities in relation to age and diabetic duration is given in Table 11 The smallness of the material only permitted a division into two age groups below and above 60 years of age. As was to be expected there was a considerably higher frequence of peripheral arterial disease in the older age group in comparison with the younger one Only one patient was under 40 years of age viz a 36 year old man with a diabetic duration of 29 years and gangrene in the foot The distribution between men and women in the two duration age groups was about the same. Percentually there was some preponderance for the women in the younger group, and for the men in the older one. There was no definite increase of the total frequence of arterial disease in the lower extremities with increasing diabetic duration. Here an unavoidable selection of the material is of consequence in that the patients with diabetic gangrene often com under the care of a surgeon However it was possible to ascertain that arterial disease of the lower extremities even in the age group below 60 could occur early after the onset of diabetes With the exception of one all the patients were over 40 years of age and, as previously pointed out it is probable that there are several pathogenetic factors in arterial disease such as e g arteriosclerosis in the large vessels in combination with the previously described changes in the small vessels.

Twenty-eight patients had gangrene located in one or more toes or in the heel and the sole of the foot. Of these 20 (71 per cent) had atrophic skin lesions on the leg Nine patients, or about one third had palpable pulses on the gangrenous foot. This is in agreement with the general conception that patients with diabetic gangrene often have retained foot pulses.

Fourteen patients with gangrene were examined with femoral angiography Six patients had normal angiograms where the vessels could be followed right down to the foot. In all these cases the foot pulses were also palpable

These 6 patients with diabetic gangrene were of special interest in that they had a normal angiogram and normal foot pulses. Their ages ranged from 45 to 69 Three of them had atrophic skin lesions on the legs Four of them had neuropathy to a slight degree with achilles and patellar areflexia and with a slight reduced sense of vibration but without reduced superficial sensibility The normal angiographic finding and the absence of severe neuropathy support the conception that a part of the origin of diabetic gangrene consists of changes in the small vessels. An impaired circulation through the large arteries may further facilitate the incidence of gangrene and prove prognostically unfavourable. As previously seen the frequence of arterial disease in the lower extremities was significantly correlated to the incidence of atrophic skin lesions But as arterial disease in the lower extremities only occurred in 14 per cent of the whole material

it could not be of any decisive significance as regards the incidence of the atrophic skin changes.

When studying the frequence of non-palpable foot pulses in arteria dorsalis pedis and arteria tibialis posterior it was found that the women in the diabetic material lacked a palpable pulse in the arteria tibialis posterior to a much greater extent than the men and that among the women the frequence of non-palpable arteria tibialis posterior was considerably greater than the frequence of non-palpable arteria dorsalis pedis. This is probably due to anatomical differences in the sexes owing to an increased amount of subcutaneous fat or oedema. The arteria dorsalis pedis is ,on account of its superficial location, more easily palpable and here, there was no difference between the sexes.

The frequence of non-palpable pulse in the arteria dorsalis pedis in patients with and without atrophic skin changes, is seen in Table 12. The total frequence of non-palpable arteria dorsalis pedis was 22 per cent. The frequence of non-palpable arteria dorsalis pedis in the group with atrophic skin changes was greater than in the group without such changes. The difference was probably significant. (χ^2 = 5.06 df = 1 0.025 > P > 0.01)

Hypertension

The frequence of hypertension has been considered to be somewhat greater in diabetics than in non-diabetics. That this is the case with diabetics with nephropathy is unmistakable. That the frequence of hypertension is greater in diabetics without nephropathy in comparison with non-diabetics is more doubtful (Lundbaek 1953).

In a large material of apparently healthy people Master (1961) found that the systolic as well as the diastolic pressure rose with increasing age and that the pressure in women was higher than in men of the same age group. The increase of blood pressure culminated in men at the age of 65 years, whereas in women it continued to rise up to the age of 70 to 74. A diabetic material that is predominantly represented by elderly people reveals a higher frequence of hypertension when compared with a normal material without this predominant representation. Very often a diabetic material is predominantly represented by elderly women. It will then contain a

TABLE 12 *The frequence of non-palpable pulse in arteria dorsalis pedis in diabetics with and without atrophic skin lesions*

	With		Without		Total	
	Cases	%	Cases	%	Cases	%
Men	22/76	29	6/40	15	27/116	23
Women	13/42	31	17/98	16	29/140	21
Total	35/118	30	21/138	15	56/256	22

TABLE 13 *The frequence of hypertension in diabetic men and women with and without atrophic skin lesions*

	With		Without		Total	
	Cases	%	Cases	%	Cases	%
Men	19/78	24	6/42	14	25/120	21
Women	20/46	44	43/111	39	63/157	40
Total	39/124	31	49/153	32	88/277	32

greater frequence of women than men with hypertension if the same lower limit for hypertension is used for men as well as women

The frequence of hypertension in the whole material was 32 per cent of the men 21 per cent had hypertension and of the women 40 per cent. The frequence of hypertension in patients with and without atrophic skin lesions is seen in Table 13 There was no significant difference in this respect between patients with and without atrophic skin changes either as regards men or women (Men $\chi^2 = 1.12$ df = 1 0.3 > P > 0.2 Yates's correction Women $\chi^2 = 0.141$ df = 1 0.8 > P > 0.7 Yates's correction) On the other hand there was a significant difference in the frequence of hypertension between men and women (χ^2 = 11.44 df = 1 P < 0.001) The difference in frequence was most pronounced in the age group exceeding 60 years. Of the 123 patients exceeding 60 years of age 14 of 41 men (34 per cent) had hypertension and 47 of 82 women (57 per cent)

The frequence of hypertension in relation to age and diabetic duration is seen in Table 14 The frequence of hypertension rose with increasing age but on the other hand there was no rise in the frequency in relation to increasing diabetic duration as will be seen from the total frequence in the lowest row and the right column of the Table Neither was there any difference in the frequence of hypertension between patients with less than and more than a 15 year diabetic duration ($\chi^2 = 0.363$ df = 1 0.6 > P > 0.5 Yates's correction)

TABLE 14 *The frequence of hypertension in diabetic in relation to age and diabetic duration*

Duration Years	< 20	%	20—39	%	40—59	%	> 60	%	Total	%
0— 4	0/11	—	0/10	—	10/26	38	27/59	46	37/106	35
5— 9	0/9	—	0/11	—	2/12	17	14/24	58	16/56	29
10—14	0/6	—	0/14	—	3/11	27	16/26	62	19/57	33
>15	—	—	8/31	26	4/13	31	4/14	29	16/58	28
Total	0/26	—	8/66	12	13/62	31	61/123	50	88/277	32

Other findings

Hereditary diabetic disposition was found in 46 per cent of the whole material the frequence being about the same for men and women. There was no significant difference in the incidence of hereditary diabetic disposition in patients with and without atrophic skin lesions (χ^2 0.959 df 1 0.6> P> 0.5)

Varicose disease was found in 14 per cent — women 16 per cent, men 11 per cent. As regards the incidence of varices there was no significant difference between patients with and without atrophic skin changes. (χ^2 0.771 df 1 0.4> P> 0.3 Yates's correction). Varicose ulcers were found in 9 patients.

Necrobiosis lipoidica was found in 9 patients — 2 men and 7 women.

Twelve patients had active or healed pulmonary tuberculosis, but tuberculous skin lesions were not diagnosed in any of the patients.

The majority of the patients were treated with insulin, or sulfonylureas and in only 9 per cent was diet alone considered to give satisfactory results.

Among the patients with atrophic skin lesions 26 per cent of the men had heavy work 38 per cent had lighter work and 36 per cent had sedentary work. The majority of the women were housewives with light or moderate manual duties.

The group with *divergent skin lesions* consisted of 16 patients whose ages varied from 18 to 85 years — 10 men and 6 women. They were evenly distributed within all the age groups, the median age being 60 years.

The lesions consisted to a great extent of isolated pigmented spots without atrophy In two or three cases there was a more diffuse pigmentation and in some cases a few small excoriations with crusts were observed.

The patients were also evenly distributed within the groups with different diabetic duration There were 4 patients in the group exceeding 15 years diabetic duration In the group with less than 5 years duration there were 6 patients In the latter group all the patients, with the exception of one were more than 40 years of age. As already seen a corresponding condition was found in the material with atrophic skin lesions. (Table 3) The patients with atrophic skin changes and with a diabetic duration of less than 5 years were more than 40 years of age the majority more than 60 years.

Retinopathy was found in more than half of the patients with divergent skin changes, and neuropathy was found in 6 of the 16 patients.

Earlier in the presentation it was seen that the atrophic skin changes gradually subsided. Atrophy and pigmentation disappeared. In some cases there was, however in the later stages a remaining slight pigmentation without atrophy

The similarity of the divergent skin lesions with older atrophic skin changes, and other similarities as regards age, diabetic duration and complications between patients with divergent skin changes and those with atrophic skin changes may probably denote that the former are an expression of older healed atrophic skin lesions and therefore may be included among them.

THE HYPERTENSION MATERIAL

The occurrence of atrophic skin lesions of the described type in non-diabetics appears to be very rare. In a normal population it is hardly ever seen. A number of patients with hypertension were examined, the reason for this being that the arteriolosclerotic vascular changes associated with hypertension might possibly give rise to similar atrophic skin lesions.

A material of 104 patients with hypertension as the principal disease were examined. The material consisted of 53 men and 51 women all of whom were admitted to the Medical Clinic in Umeå.

On the whole, the age distribution was comparable to that of the diabetic material although somewhat more evenly distributed and lacked the double curve peaks of the latter. The median age was somewhat lower in the hypertension material than in the diabetic one. The median age for men was 52.6 years and that for women was 53.1 years. Corresponding figures for the reported diabetic material was 50.0 and 60.3 years respectively. The youngest patient with hypertension was 19 and the oldest 77 years of age.

Manifest diabetes was not diagnosed in any of the patients. In 8 patients (8 per cent) there was a hereditary diabetic disposition. In the diabetic material the corresponding figure was 46 per cent.

Nine patients — 6 men and 3 women — had similar atrophic skin lesions on the legs to those observed in diabetics. The ages of these patients varied from 45 to 63 years and they had had hypertension from 3 to 17 years. Two of them had malignant hypertension. A diabetic heredity was found in 1 patient.

In 7 of the 9 patients the skin lesions could not be differentiated from the diabetic» ones. The two remaining patients had somewhat divergent changes, and only some single brownish pigmented skin lesion with insignificant atrophy was found.

In 6 of the 7 cases with typical lesions an oral glucose tolerance test was performed in a manner that will be described in Chapter V. Two patients had a diabetic curve that did not return to the fasting value three hours later. Four patients hade a glucose tolerance curve that returned to the fasting value within 3 hours, but which rose maximally to more than 200 mg per cent (221—250 mg per cent). One year later one of these patients developed a manifest diabetes which confirmed the pathogenicity in the 3 other curves. The two patients with atypical skin lesions had glucose tolerance curves which returned to the fasting value within 3 hours and which did not exceed 200 mg per cent.

The patients with skin changes corresponding to those observed in diabetics had thus a diminished glucose tolerance and were assessed as latent diabetics. One of them later developed a manifest diabetes.

In view of the possibility of there being a traumatic origin to the atrophic skin changes, a group of 50 active football players whose ages varied from 17 to 40 years, were examined during the current football season. As these players are subject to repeated leg injuries it was thought that they might reveal a high frequence of traumatic skin changes. No atrophic skin lesions of the described

type were found. Many of the players had superficial excoriations and some of them had diffuse pigmentations. A 70 year old man, a so-called old boy who had not taken an active part in the match, had atrophic skin lesions of the described type on the legs, but had also diabetes.

In a woman with systemic lupus erythematosus (SLE) and without signs of a manifest diabetes, brownish atrophic skin lesions were observed on the lower legs. However no glucose tolerance test was performed owing to her poor condition.

In connection with this finding an examination was made of a material of patients with collagen diseases including SLE, dermatomyositis and polyarteritis nodosa with regard to atrophic skin lesions on the legs. Vascular changes have also been described in several of these diseases (cf Angevine 1961 Mackay and Burnet 1963) The material consisted of 30 patients. Only one patient more, a 40 year old man with SLE, had atrophic skin changes on the legs. He had, however a pathologic glucose tolerance curve and later developed a manifest diabetes.

CHAPTER V

ATROPHIC SKIN LESIONS IN THE LOWER EXTREMITIES OF PATIENTS WITHOUT MANIFEST DIABETES

One of the incentives to a systematic study of the question relating to the atrophic skin lesions in the lower extremities in diabetics was as already mentioned in the introduction the observation that these lesions could also occur in middle aged and old patients without glucosuria and that an oral glucose tolerance test in these cases often revealed a so-called latent diabetes The observation denoted that the occurrence of these lesions was partly associated with a change of the glucose metabolism in a diabetic direction and partly could be an early and directly obvious sign of diabetes.

Those patients with the aforementioned skin lesions without glucosuria and an earlier diagnosed diabetes were subjected to examination with the oral glucose tolerance test

The glucose tolerance test may be performed in many different ways (Conn 1940 Mosenthal and Berry 1950 Fajans and Conn 1954 Ikkos and Luft 1957) In this investigation the oral glucose tolerance test was chosen. Statements as to where the limit goes between a normal and reduced glucose tolerance vary among different authors and is inter alia due to the choice of method for glucose determination and if the capillary or venous blood have been examined, and also the age of the control material (Jackson 1952 Chesrow and Bleyer 1954 Conn and Arbor 1958 Crombie and FitzGerald 1962 Oberdisse et al 1962) As a rule the glucose tolerance is interpreted on the basis of the blood sugar value 1 5 to 2 hours after the ingestion According to another interpretation the blood sugar should have returned to the fasting value within a given time, usually from 2 to 2 5 hours. For old people the glucose tolerance curve is said to return within a period of 3 hours after the loading (Mosenthal and Berry 1950 Chesrow and Bleyer 1954 1957 Hecht et al 1961) As the glucose tolerance is thus diminished in elderly people, it is expedient when interpreting the glucose tolerance test to have a control material comparable in age.

MATERIAL

The patient material with atrophic skin lesions, but without glucosuria or previously known diabetes was not obtained by a systematic study of a certain patient material and does not therefore permit conclusions concerning the frequence of this symptom The material was obtained more or less, by incidental observations of these lesions in patients who were admitted to the Medical Clinic and also as a result of the search for the lesions in patients examined in the Out Patient Clinic.

The material consisted of 17 patients whose ages ranged from 40 to 83 — median age 58 years, all of whom were admitted to the clinic at the time of examination. All of them had typical atrophic skin lesions on the lower parts of the legs, but without glucosuria or earlier diagnosed diabetes. Three patients had slight polyneuritis corresponding to that found in diabetics. None of the patients had noticeable diabetic vascular changes such as retinopathy or nephropathy. Six of the patients had close relatives with diabetes. All the patients were examined with the oral glucose tolerance test.

The control material consisted of 46 inpatients comparable in age (40 to 78 years — median age 53) without diabetes and diabetic heredity. None of the patients was bedridden at the time of examination.

Of those patients with atrophic skin lesions 8 had diagnosed cardiosclerosis and 3 of these had a previosly diagnosed cardiac infarction. None of the patients had collagen disease. The majority of the comparative material had slight arthrosis and spondyloarthrosis for which they were receiving treatment.

METHOD

The oral glucose tolerance test was performed after the patients were put on a rich carbohydrate diet (>300 g carbohydrate) for 3 days. On the morning following a 12 hours fast 100 g of glucose was ingested. Capillary blood for blood glucose assessment was taken partly before loading (fasting value) and partly every half hour after the ingestion during a period of 4 hours. The blood sugar was assessed according to a modification of the glucose-oxidase method (Keston 1956, Teller 1956, Levin and Linde 1962). The blood sugar values obtained were plotted on a mm-square paper with the time along the abscissa and the blood sugar values along the ordinate. The resultant surface was determined planimetrically and the value obtained represented a measure of the glucose tolerance. The significance test was carried out according to Fisher's t formula for testing the difference between means (Guildford 1950).

Unfortunately the blood sugar method was altered during the investigation period by changing the precipitation of the blood protein which gave somewhat higher blood glucose values. The material was thus divided into two series (Series I and II) before and after the re arrangement. Series I included 10 patients with atrophic skin lesions and 19 patients as comparative material. Series II included 7 patients with atrophic skin changes and 27 patients as comparative material.

RESULTS

The mean curve for the control patients in both series in seen in Fig. 3. Both curves return to the fasting value within 3 hours. The curves rise maximally up to 152 and 159 mg per cent respectively.

Of the glucose tolerance curves for the 10 patients in Series I with atrophic skin lesions 6 had not returned to the fasting value three hours later. Of the 4 patients whose curves had returned to the fasting value within 3 hours, 2 of them had so-

called lag curves with a maximal rise to 200 mg per cent. Of the glucose tolerance curves for the 7 patients with *atrophic skin changes in Series II* all excepting one had not returned to the fasting value within 3 hours The remaining patient had a lag-curve with a maximal peak at 215 mg per cent

The mean value of the planimetrically estimated surface for the glucose tolerance curves *in Series I* was greater in patients with atrophic skin lesions in comparison with the controls. The difference was significant (t = 2 661 df = 27 0 02> P> 0 01) In Series II also the mean value of the planimetrically assessed surface was greater in patients with *atrophic skin lesions when compared with* the controls The difference was significant (t = 2 214 df = 32 0 05> P> 0 025)

The patients with atrophic skin lesions and without glucosuria thus differed from a comparative material of corresponding age *in that they had a statistic*ally proved change of the oral glucose tolerance. The oral glucose tolerance had changed in a diabetic direction

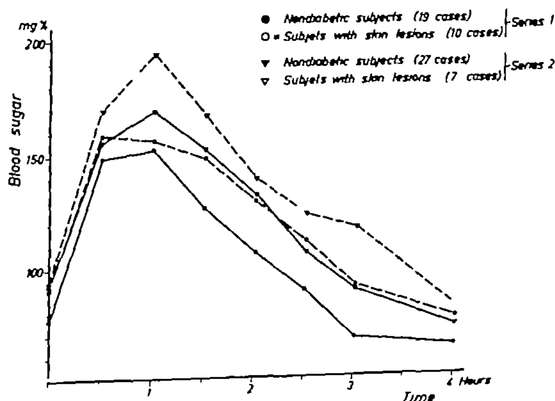


FIG 3 *Oral glucose tolerance curve from patient with atrophic skin lesion and without known diabet s compared to non-diabetic control f the same age*

CHAPTER VI

HISTOPATHOLOGICAL FINDINGS

Histopathological studies of the vascular lesions in the skin muscles and nerves in the lower extremities of diabetics were conducted by Goldenberg et al (1959). Without a previous knowledge of the clinical history they examined specimens from 152 amputations from the lower extremities (92 diabetics and 60 non-diabetics) with reference to the small vessels. Only those cases that showed a combination of endothelial hypertrophy and hyperplasia and PAS-stained structures in the walls of the small vessels were classified as »diabetic». Successive sections were stained with hematoxylin-eosine periodic acid Schiff (PAS) colloidal iron of Hale modified by Rinehart and Abul-Haj (1951) and with Verhoeff—van Gieson. The Abul-Haj technique was used for separating arteriosclerotic structures from non-arteriosclerotic ones. There was only 2 per cent diagnostical error in the 152 specimens diagnosed as diabetic instead of non-diabetic. The digital artery was the largest in which the diabetic lesion could be distinguished. The »diabetic» vessel changes were found in 92 per cent of the diabetics, and in 8 per cent of the non-diabetics. They were found in 97 per cent of the vasa vasorum, in 73 per cent of the vasa nervorum and in 63 per cent of the skin vessels including the capillaries. In about half the cases there was no clinical evidence of either retinopathy or nephropathy. The authors concluded that the lesions demonstrated in the small vessels of diabetics could be distinguished from arterio- and arteriolosclerosis. Thus the diabetic lesions were characterized by endothelial proliferation in which were deposited PAS-positive fibrills in a reticulated pattern and not stained with colloidal iron (Abul-Haj). The most striking changes were found in the arterioles and capillaries.

A confirmatory study of the lower extremities of diabetics was conducted by Pedersen and Olsen (1962). They laid stress upon the often uneven and segmental distribution of the lesions in the small vessels.

Similar changes of the small vessels in the skin of the forearm were demonstrated by Handelsman et al. (1962) in 8 of 12 diabetics of the juvenile and maturity-onset type without other clinical vascular changes and with diabetes of relatively recent onset. Of 6 patients with retinopathy and nephropathy all had distinctive lesions in the small vessels.

In an electron microscopic study on skin from the pulpa of 4 diabetics Aagenaes and Moe (1961) found the capillary wall much thicker when compared with the non-diabetic controls. The widening

was due to a material similar in density and structure to the basement membrane.

MATERIAL

The material consisted of skin specimens from the lower parts of the legs in 11 patients with diabetes. In 4 patients the skin specimens were obtained at autopsy and were the same as those studied by microangiography. The remaining specimens consisted of skin biopsies. In all these cases the specimens consisted of the previously described atrophic skin changes with adjacent normal skin.

The comparative material consisted of 5 patients. Four of them were autopsies. The remaining specimen was that of an amputated leg from a patient with pelvic sarcoma. The skin specimens from 3 of the patients were the same as those used for the microangiographic examination. Clinical data of the material is presented in Table 15.

TABLE 15. *The diabetic material and the non-diabetic control material used for histopathological examination of skin from the lower extremities*

	Patient		Age (Yr.)	Diabetics Duration of diabetes	Complications	Diagnosed as diabetics
1	I. J.	female	32	23 yr	Rp., np., neup.	+
2	K. A.	male	36	20 yr	Rp., np.	—
3	N. L.	male	61	5 yr	Rp.	+
4	E. M.	male	64	6 yr	Rp., np., neup.	+
5	S. L.	male	40	21 yr	Rp., neup.	+
6	M. P.	male	40	15 yr	Rp., np., neup.	+
7	V. G.	male	48	Latent diab.	—	+
8	A. H.	male	59	2 m.	—	—
9	E. N.	male	59	31 yr	Rp., neup.	+
10	G. E.	male	63	13 yr	Rp., neup.	+
11	E. B.	male	66	1 w	—	+
	Patient		**Age (Yr.)**	**Non-diabetic controls Disease**		
1	B. E.	male	21	Acute myocarditis		—
2	S. E.	male	53	Malignant hypertension		—
3	F. L.	male	59	Cancer ventriculi		—
4	E. L.	female	71	Acute pancreatitis		—
5	G. J.	male	37	Pelvic sarcoma		—

1—4 of the diabetics and 1—4 of the controls were autopsy specimens and 5—11 of the diabetics were biopsies. Control No. 5 was a specimen from an amputated lower extremity. Rp. denotes diabetic retinopathy, np. denotes diabetic nephropathy and neup. denotes diabetic neuropathy.

METHOD

The skin specimens were fixed in 10 per cent formalin and embedded in paraffin. In order to search for the vascular changes mentioned above successive sections from each paraffin block were stained with haematoxylin-eosine periodic acid-Schiff Rinehart and Abul-Haj (1951) colloidal iron staining and Gomori v Gieson staining. One of the blocks from each of the previously described microangiographic specimens was serie-sectioned and stained in the same way Specimens from the biopsy material were also stained with regard to the presence of hemosiderin (Berlin blue reaction) and melanin (Masson) in order to gain some idea of the type of pigment in the atrophic skin changes.

The slides were examined without a previous knowledge of the clinical history and then re-examined with the clinical key It was assumed that the cases with diabetes would show a combination of endothelial proliferation and an increase of PAS-stained material especially in the capillaries and small vessels in the stratum papillare as pointed out by Handelsman et l. (1962) They used a 4-graded scale for the alterations and found that the 3rd and 4th degrees of alteration were chiefly present in diabetics.

RESULTS

All told there were two diagnostic errors in the specimens from the 11 diabetic patients classified as non-diabetic (Table 15). One was a 36 year old man with a diabetic duration of 20 years and with both retinopathy and nephropathy The other was a newly discovered diabetes in a 59 year old man without clinical diabetic complications

In the remaining cases the vascular changes were often pronounced. They appeared best in the PAS-staining. The most advanced changes were chiefly found in the superficial dermis — stratum papillare. The capillaries often showed thickened walls, sometimes with obliteration of the lumen. On the other hand, the large vessels in the deeper layers of the dermis did not show pronounced changes as often and consequently they were not as well correlated to the diabetic disease as the small vessels in the papillary layer of the dermis. The 5 patients in the comparative material were all with regard to the skin specimens, diagnosed as non-diabetics. The superficial vessels in the dermis were throughout without corresponding changes, or with only insignificant PAS-deposits in the walls.

When the slides from the two »erroneously diagnosed diabetic patients were compared with the control specimens, after the clinical diagnosis was known, no definite difference was found between them as regards the vascular changes and consequently there was no reason to change the diagnosis.

The atrophic skin lesions consisted of atrophy of the epidermis sometimes with indicated hyperkeratosis. A similar but not so extensive atrophy of the epidermis could sometimes be seen in the skin outside the atrophic areas. In the dermis there was slight fibrosis with a change in the collagenous structure. In some cases the dermis showed edema with separation of collagen fibers which, in these

cases, was probably a question of early skin lesions

The occurrence of vessels in the dermis varied widely and the vessel changes and degree of change did not deviate from that of the adjacent skin (Fig 15 p 73) Relatively normal vessels, especially in the papillary layer could be seen side by side with distinctly changed vessels (Fig 16 p 73) On some occasions a slight round cell infiltration was observed round the vessels. The Abul Haj stained sections were negative as regards the superficial vessels in the dermis indicating the absence of arteriolosclerotic changes The evaluation was complicated in that a number of structures in the dermis are normally coloured in the Abul-Haj staining No definite changes were seen in the subcutis. On certain occasions a slight increase of fibrous tissue was found.

The amount of melanin in the atrophic skin lesions could be increased within the basal cell layer of the epidermis, but similar changes were also seen in the adjacent skin and also in non-diabetic skin In the dermis of the lesions there was often seen an insignificant quantity of hemosiderin.

The brown colour of the atrophic lesions was probably due to the melanin being more visible as a result of the atrophy of the epidermis and an increased quantity of hemosiderin In connection with abundant vascularization this may be decisive for the colour (See page 46)

CHAPTER VII

MICROANGIOGRAPHIC STUDIES

With the aid of microangiography it is possible to visualize the blood vessels as far down as to the capillaries. In contact microangiography the object is placed in close contact with a fine-grained photographic emulsion during exposure. The object is reproduced in natural size on the microradiogram. The primary microradiogram is studied in the microscope or enlarged according to usual photographic procedures. The maximal degree of enlargement is inter alia dependent upon the geometric definition and the resolution of the photographic emulsion. Stereo-microangiography affords a better conception of the internal relation of the vessels (cf Bellman 1953 Odén et al 1958)

According to current conceptions of the blood supply of the skin the arterial vessels f om the subcutis form a plexus in the transition to the dermis. From here the vessels penetrate the dermis and form a new subpapillary network parallel to the surface of the skin, from which small vessels finally supply the papillae in the shape of one or more capillary loops in each papilla In a corresponding manner the veins form draining plexuses in the skin. A shunting of the blood may partly take place through coarse arteriovenous shunts and partly through so-called preferential channels (cf Spalteholz 1927 Montagna 1962).

METHOD

The investigation was carried out on autopsy material. The arteria and vena femoralis were cannulized immediately below the groin. The leg was filled with contrast by means of the artery catheter The contrast consisted of Micropaque (Damancy & Co) The suspension was warmed to body temperature. Twenty per cent of Micropaque in isoton saline followed by 20 per cent of Micropaque in 3 per cent of gelatin solution was injected. The injection was done rapidly with a 20 cc glass syringe. In order to obtain a better filling of contrast a tourniquet was placed around the leg immediately below the site of cannulization with a free passage through the catheters. After the contrast injection the skin was cooled in running water whereupon skin specimens 2 2 cm. in size were excised with the subcutis attached The skin lesions had been previously marked with Indian ink on the skin surface The excised specimens were mounted on a cork disc and fixed in 10 per cent of formalin and later embedded in paraffin.

For the microangiographic examination a Machlett X-ray therapy tube was used, Type AEG 50, with a 1 mm beryllium window and a wolframanode. For the exposure 20 mA and 15 kV were used. The photographic emulsion was Kodak M. R. Plate which has a resolut

ion of 500—1000 lines per mm. To avoid light exposure the plates were wrapped in 12 micron thick aluminium foil. The objects were stereo-microradiographed in toto and vertical to the skin surface. The specimens were then cut into 3—4 mm thick sections vertical to the skin surface. These sections were stereo-microradiographed vertically to the cut surface. This projection was found most suitable for the study of the superficial skin vessels.

Apart from the contrast in the paraffin-embedded specimens having decreased somewhat in diameter no difference was seen in the microangiogram before and after the embedding. At histological examination the contrast was found as far out as to the capillaries in the papillary layer of the skin.

MATERIAL

The material consisted of skin from 4 diabetic patients, 3 of whom had atrophic skin lesions on the legs. The ages of the patients ranged from 34 to 64 years, all of whom had long-term diabetic complications. All told 17 skin specimens with atrophic skin lesions were examined and 5 specimens of clinically normal skin. As a comperative material the skin from 4 non-diabetic patients (20 to 52 years of age) was examined, 3 of whom had chronic nephritis, hypertension and acute myocarditis respectively. The fourth specimen was that of an amputated leg from a patient with pelvic sarcoma. All together the comparative material consisted of 6 skin specimens.

RESULTS

The microangiograms of skin from the non-diabetics revealed an abundance of vessels and the capillaries in the papillary layer of the dermis were often observed (Fig. 4, p. 69). As a rule the distribution of the vessels was not so schematic as previously outlined and more often than not no demarcated arterial plexuses were observed. No contrast deposits signifying extravasation of contrast were found.

The skin from the diabetics presented a different picture to that from the non-diabetics. The macroscopically more diabetic skin was rich in larger vessels in the lower parts of the dermis. The intermediate and superficial dermis was, however, very poor in vessels in comparison to the skin in the control material. The small vessels were irregular in shape and varied in calibre, and the capillaries in the papillary layer could seldom be observed. In 4 specimens there were some small spherical contrast deposits at different depths in the dermis attached to the small vessels. It was difficult to decide if they consisted of extravasal contrast or were of another nature, e.g. microaneurysms.

As regards the vessel supply the atrophic skin lesions often presented a different picture to that of the adjacent skin. In 9 of the 17 skin lesions there was a greater abundance of small vessels in the dermis than in the nearby skin (Fig. 5, p. 69). A great number of small vessels and capillaries radiating from the larger and deeper vessels were often observed to spread fan-wise towards the surface of the skin. No interruption of the contrast in the larger vessels of the

dermis could be seen as a sign of an occlusion.

The investigation thus proved that a good half of the examined atrophic skin lesions in diabetics were richer in capillaries than in the adjacent skin. The macroscopically intact diabetic skin especially in the superficial parts of the dermis was not so rich in vessels as the non-diabetic skin in the comparative material.

ion of 500—1 000 lines per mm. To avoid light exposure the plates were wrapped in 12 micron thick aluminium foil The objects were stereo-microradiographed in toto and vertical to the skin surface The specimens were then cut into 3—4 mm thick sections, vertical to the skin surface. These sections were stereo-microradiographed vertically to the cut surface. This projection was found most suitable for the study of the superficial skin vessels

Apart from the contrast in the paraffin-embedded specimens having decreased somewhat in diameter no difference was seen in the microangiogram before and after the embedding At histological examination the contrast was found as far out as to the capillaries in the papillary layer of the skin

MATERIAL

The material consisted of skin from 4 diabetic patients 3 of whom had atrophic skin lesions on the legs The ages of the patients ranged from 34 to 64 years, all of whom had long term diabetic complications All told 17 skin specimens with atrophic skin lesions were examined and 5 specimens of clinically normal skin As a comperative material the skin from 4 non-diabetic patients (20 to 52 years of age) was examined 3 of whom had chronic nephritis, hypertension and acute myocarditis respectively The fourth specimen was that of an amputated leg from a patient with pelvic sarcoma All together the comparative material consisted of 6 skin specimens.

RESULTS

The microangiograms of skin from the non-diabetics revealed an abundance of vessels and the capillaries in the papillary layer of the dermis were often observed (Fig 4 p 69) As a rule the distribution of the vessels was not so schematic as previously outlined and more often than not no demarcated arterial plexuses were observed. No contrast deposits signifying extravasation of contrast were found.

The skin from the diabetics presented a different picture to that from the non-diabetics The macroscopically intact diabetic skin was rich in larger vessels in the lower parts of the dermis The intermediate and superficial dermis was, however very poor in vessels in comparison to the skin in the control material The small vessels were irregular in shape and varied in calibre and the capillaries in the papillary layer could seldom be observed In 4 specimens there were some small spherical contrast deposits at different depths in the dermis attached to the small vessels. It was difficult to decide if they consisted of extravasal contrast or were of another nature e. g microaneurysms.

As regards the vessel supply the atrophic skin lesions often presented a different picture to that of the adjacent skin In 9 of the 17 skin lesions there was a greater abundance of small vessels in the dermis than in the nearby skin (Fig. 5 p 69) A great number of small vessels and capillaries radiating from the larger and deeper vessels were often observed to spread fan-wise towards the surface of the skin No interruption of the contrast in the larger vessels of the

the lower extremities (Rotwit et al. 1953 Scott 1961). Moreover the disappearance rate in subcutaneous tissue is much slower than that of the skin (Wisham and Yalow 1952) and it is well known that skin has a greater number of blood vessels than subcutaneous tissue.

It may be assumed that some of the injected isotope e. g. Na^{24} is cleared by the lymph vessels. By measuring the radioactivity in the ductus thoracicus in the dog, Stone and Miller (1949) found that only about 1 per cent of Na^{24} locally injected in the muscle was carried away by lymphatic drainage, and that the predominating part was cleared by the blood vessels.

The slow disappearance of I^{131} labeled albumin from subcutaneous tissue in human beings, in contrast to the rapid disappearance of radioiodide from the same tissue indicated that the former substance might be cleared by lymphatic drainage. (Hollander et al 1961)

Local disappearance studies on diabetics are rare. Ries et al. (1960) found a decreased subcutaneous disappearance rate in diabetics as compared with non-diabetics. The low disappearance rate was said to be correlated to the diabetic duration and thus an expression of a general microangiopathy

Theoretical technical as well as medical aspects of disappearance measurements have been extensively described by Odeblad and Westin (1959)

MATERIAL AND METHOD

An investigation was carried out with the aid of I^{131} (half life 8.1 d.) and Na^{24} (h.l. 15.1 h.) in diabetic patients with atrophic skin changes on the legs. The isotope was intradermally injected in the atrophic skin lesion and, by way of comparison, in adjacent normal skin. In a similar manner a number of patients were studied with atrophic scars of a definite traumatic genesis. All the patients were hospitalized.

A scintillation counter (NaI (Tl) crystal) with a pulse height analyzer was used for the measurements. From the analyzer the pulses were registered both by a scaler and a ratemeter with a pen recorder The time constant for the ratemeter was 0.5 seconds. The detector was surrounded by a lead shield of at least 4.5 cm in thickness, and was supplied with a cylindrical collimator 2.5 cm in diameter and 6.0 cm in length. The pulse height analyzer was adjusted to the 364 keV photopeak of I^{131} and the 1.38 MeV photopeak of Na^{24} The width of the channel was about 60 keV The background was about 100 counts per minute (cpm) and the injected activity about 1 microcurie gave 5—6.000 cpm.

In a special series of tracer tests the two isotopes were measured simultaneously by using two pulse height analyzers with ratemeters. In this series the channel widths of the two pulse height analyzers were adjusted to give about the same counting rates for I^{131} and Na^{24} The chemical form for I^{131} was sodiumiodide and for Na^{24} sodiumchloride both being diluted in isotonic saline.

A micrometer syringe was used in order to inject small volumes. The accuracy of the volumes delivered was stated to be ± 0.00005 ml. One complete revolution of the micrometer head delivered a volume of 0.01 ml but the head was graded in fiftieths and the smallest vol-

CHAPTER VIII

THE DISAPPEARANCE RATE OF RADIOACTIVE IODIDE (I^{131}) AND SODIUM (Na^{24}) IN THE ATROPHIC SKIN LESIONS

After a local injection of radioactive isotopes the disappearance of the isotope may be studied by in vivo measurement. Small radioactive ions may after interstitial injection be expected to disappear preferably through diffusion and the disappearance rate may give elucidating information about the local circulation in the tissue.

The basis for this procedure was mathematically and experimentally analyzed by Kety (1948 1949) He used Na^{24} and postulated that the disappearance or clearance of the isotope from the site of injection followed a single exponential function It was also suggested that the disappearance rate yielded a rough index of the effective circulation.

Disappearance studies have been carried out on different tissues such as the muscle the subcutaneous tissue and the skin (Kety 1948 Elkin et al 1948 Wisham et al 1951 Barron et al 1951 Braithwaite et al 1951 McGirr 1952 Hanser et al 1961) Disappearance studies of muscle and subcutis have been carried out to examine the circulation in the lower extremities in cases of arterial disease (Walder 1953 Philipp and Ruf 1953 Pabst 1955)

A relatively good correlation has been found between the blood flow as judged by plethysmography and the disappearance rate in muscle under different conditions (Rapaport et al. 1952)

No practical difference in the disappearance rate between Na^{24} and I^{131} has been found (Hyman et al. 1950 Bauer et al 1953) For practical reasons I^{131} has often been preferable because of its longer half life

Clearance studies on the skin have chiefly been carried out in the field of plastic surgery for the evaluation of the circulation in grafts (Barron et al 1951 Braithwaite et al 1951 Hanser et al 1961)

Only a few studies on the conditions in dermatologic diseases have been carried out to gain some idea of the local circulation (Bettley and Fairburn 1957 Blocker et al. 1957)

It has been pointed out that the total circulation in the skin could not be measured by this method. This is due to the disappearance rate being dependent upon the capillary blood flow and not being influenced by the flow through the arterio-venous shunts or by-pass channels in the skin. The disappearance rate is practically an expression of the nutritive circulation (Kety 1953 Walder 1953) In agreement with this it has been established that skin areas rich in capillaries such as the face have a more rapid clearance rate than that found in areas poor in capillaries e g the abdomen and

the lower extremities (Rotwitt et al. 1953 Scott 1961). Moreover the disappearance rate in subcutaneous tissue is much slower than that of the skin (Witham and Yalow 1952) and it is well known that skin has a greater number of blood vessels than subcutaneous tissue.

It may be assumed that some of the injected isotope e. g. Na^{24} is cleared by the lymph vessels. By measuring the radioactivity in the ductus thoracicus in the dog, Stone and Miller (1949) found that only about 1 per cent of Na^{24} locally injected in the muscle was carried way by lymphatic drainage and that the predominating part was cleared by the blood vessels.

The slow disappearance of I^{131} – labelled albumin from subcutaneous tissue in human beings, in contrast to the rapid disappearance of radioiodide from the same tissue, indicated that the former substance might be cleared by lymphatic drainage. (Hollander et al 1961).

Local disappearance studies on diabetics are rare. Ries et al. (1960) found a decreased subcutaneous disappearance rate in diabetics as compared with non-diabetics. The low disappearance rate was said to be correlated to the diabetic duration and thus an expression of a general microangiopathy.

Theoretical, technical as well as medical aspects of disappearance measurements have been extensively described by Odeblad and Westin (1959).

MATERIAL AND METHOD

An investigation was carried out with the aid of I^{131} (half-life 8.1 d.) and Na^{24} (h.l. 15.1 h.) in diabetic patients with atrophic skin changes on the legs. The isotope was intradermally injected in the atrophic skin lesion and, by way of comparison, in adjacent normal skin. In a similar manner a number of patients were studied with atrophic scars of a definite traumatic genesis. All the patients were hospitalized.

A scintillation counter (NaI (Tl) crystal) with a pulse height analyzer was used for the measurements. From the analyzer the pulses were registered both by a scaler and a ratemeter with a pen recorder. The time constant for the ratemeter was 0.5 seconds. The detector was surrounded by a lead shield of at least 4.5 cm in thickness, and was supplied with a cylindrical collimator 2.5 cm in diameter and 6.0 cm in length. The pulse height analyzer was adjusted to the 364 keV photopeak of I^{131} and the 1.38 MeV photopeak of Na^{24}. The width of the channel was about 60 keV. The background was about 100 counts per minute (cpm) and the injected activity about 1 microcurie gave 5—6.000 cpm.

In a special series of tracer tests the two isotopes were measured simultaneously by using two pulse height analyzers with ratemeters. In this series the channel widths of the two pulse height analyzers were adjusted to give about the same counting rates for I^{131} and Na^{24}. The chemical form for I^{131} was sodiumiodide and for Na^{24} sodiumchloride both being diluted in isotonic saline.

A micrometer syringe was used in order to inject small volumes. The accuracy of the volumes delivered was *stated to* be ± 0.00005 ml. One complete revolution of the micrometer head delivered a volume of 0.01 *ml* but the head was graded in fiftieths and the smallest vol-

ume delivered thus corresponded to one 0 0002 ml The needle a fine gauge steel needle No 26 was cemented to the syringe to eliminate leakage. The syringe was siliconized to avoid back flow leakage.

Somewhat varying volumes were used for the intradermal injections during the examinations But however the same volume was used on each separate occasion The injected volume varied from 0 03 to 0 002 ml and contained about 1 microcurie of the radioactive material The larger volumes were, above all conditioned by the low activity of the Na^{24} on arrival from the pile

The examination was performed with the patient in a recumbent position The investigation commenced after a 30 minute rest during which the background activity was registered. The temperature in the investigation room was practically constant 22—23 C. Blair et al (1959 1960) has shown that there is no appreciable vasoconstrictor or vasodilator tone in the skin vessels of the leg at this temperature and when the patient is comfortably warm.

Intradermal injections were given alternating in the atrophic skin lesion and in the normal skin The aperture of the collimator was placed a few mm above the site of injection The measurement was started about one minute after the injection

The activity was registered with the aid of the recorder and as an extra check by counting the number of impulses for a period of one minute every other minute As a rule, the measurements were completed 15 minutes later In a corresponding manner a new intradermal injection was given adjacent to the first point of measurement. Prior to this injection the body background was measured at the point for the new one The distance between the points of measurement was about 10 cm.

When the measurement values had been corrected for the background they were plotted against time on a semilogarithmic paper where these values were placed in accordance with the logarithmic scale and the time according to the linear scale. The disappearance constant, the k value was then calculated. The k value was obtained according to the formula

$$k = \frac{\log C_1 - \log C_2}{0\,4343\ (t_2 - t_1)}$$

Where C_1 and C_2 are the counts per minute at t_1 and t_2 respectively (Kety 1949)

A simpler way of obtaining the k value is to decide the time to decrease the activity by half the biological half time (T ½) The k value may then be obtained according to the formula

$$k = \frac{0\,693}{T\ ½}$$

where 0.693 is the natural logarithm for 2

For the significance test of the paired series Fisher s t formula for testing the difference between correlated pairs of means was used (Guilford 1950)

For the significance test of the nonpaired series Fisher s t formula was used for the difference between uncorrelated pairs of means (Guilford 1950)

PREPARATORY STUDIES

In order to ascertain if the somewhat varying volumes used had any effect on

the disappearance rate of the isotope, tests were carried out on 3 patients. In each of them the intradermal disappear ance rate in normal skin of the leg was assessed at 0.002, 0.01 and 0.03 ml. intradermally injected I^{131}. No difference in the disappearance rate was recorded in the three patients. The mean k-values were 0.111 (0.082 – 0.133), 0.122 (0.091 – 0.157) 0.115 (0.091 – 0.139) for the three different volumes respectively.

In order to ascertain if there was any difference in the disappearance rate between I^{131} and Na^{24} a mixed isotope solution with both isotopes was injected. The pulse height analyzer was so adjusted that the counting rate was about the same for both isotopes. The test was carried out on 5 patients, 3 of whom had diabetes. The disappearance rates for both isotopes were almost identical. (Table 16).

In order to ascertain if there was any difference in the disappearance rate between two adjacent deposits of intradermally injected I^{131} 15 patients were examined, 2 of whom had diabetes with durations of 2 and 5 years and the others were non-diabetics. All of them were healthy without signs of cardiovascular disease. The injections were given 8 to 10 cm. from each other in a proximal-distal direction on the front of the lower part of leg, and alternatingly the proximal and distal point were first injected and measured. The results are given in Table 17. There was no statistical difference in the disappearance rate between the two points measured. ($t = 0.282$, $df = 14$, $0.8 > P > 0.7$)

RESULTS

In a series of 17 patients with diabetes and with atrophic skin lesions on the lower legs the disappearance rate of I^{131} was assessed partly in the atrophic skin lesion and partly in the intact adjacent skin. (Table 18). The ages and diabetic duration of the patients are given in the Table. The mean k-value of the intact skin was 0.071. There was no difference in the disappearance rate for patients below or above the median age in the group. Nor was there any difference in the disappearance rate between patients who had a diabetic duration of less or more than 15 years.

TABLE 16. *Disappearanc ate of I^{131} and Na^{24} in common intradermal deposit in the normal skin of the leg.*

	I^{131}		Na^{24}	
	T ½	k-value	T ½	k-value
1 T K. 20 yr male	12.2	0.057	12.2	0.057
2 C W 23 yr male	8.6	0.081	8.6	0.081
3 A H. 37 yr female	22.0	0.031	21.0	0.033
4. A. E. 38 yr male.	24.0	0.029	23.0	0.030
5 E T 63 yr female.	27.0	0.026	26.0	0.027
Mean	18.8	0.045	18.2	0.046

*) Diabetics with duration of diabetes 15, 31 and 12 years respectively

ume delivered thus corresponded to one 0 0002 ml The needle a fine gauge steel needle No 26 was cemented to the syringe to eliminate leakage The syringe was siliconized to avoid back flow leakage.

Somewhat varying volumes were used for the intradermal injections during the examinations. But however the same volume was used on each separate occasion The injected volume varied from 0 03 to 0 002 ml and contained about 1 microcurie of the radioactive material The larger volumes were, above all conditioned by the low activity of the Na^{24} on arrival from the pile

The examination was performed with the patient in a recumbent position The investigation commenced after a 30 minute rest during which the background activity was registered The temperature in the investigation room was practically constant 22—23° C. Blair et al (1959 1960) has shown that there is no appreciable vasoconstrictor or vasodilator tone in the skin vessels of the leg at this temperature and when the patient is comfortably warm

Intradermal injections were given alternating in the atrophic skin lesion and in the normal skin The aperture of the collimator was placed a few mm above the site of injection The measurement was started about one minute after the injection

The activity was registered with the aid of the recorder and as an extra check, by counting the number of impulses for a period of one minute every other minute As a rule the measurements were completed 15 minutes later In a corresponding manner a new intradermal injection was given adjacent to the first point of measurement. Prior to this injection the body background was measured at the point for the new one The distance between the points of measurement was about 10 cm

When the measurement values had been corrected for the background they were plotted against time on a semilogarithmic paper where these values were placed in accordance with the logarithmic scale and the time according to the linear scale. The disappearance constant the k value was then calculated. The k value was obtained according to the formula

$$k = \frac{\log C_1 - \log C_2}{0\,4343\ (t_2 - t_1)}$$

Where C_1 and C_2 are the counts per minute at t_1 and t_2 respectively (Kety 1949)

A simpler way of obtaining the k value is to decide the time to decrease the activity by half the biological half time ($T\ 1/2$) The k value may then be obtained according to the formula

$$k = \frac{0\,693}{T\ 1/2}$$

where 0 693 is the natural logarithm for 2

For the significance test of the paired series Fisher's t formula for testing the difference between correlated pairs of means was used (Guilford 1950)

For the significance test of the non-paired series Fisher's t formula was used for the difference between uncorrelated pairs of means (Guilford 1950)

PREPARATORY STUDIES

In order to ascertain if the somewhat varying volumes used had any effect on

On the other hand, there was a distinct difference in the disappearance rate between the atrophic skin lesions and the adjacent intact skin i. e. the disappearance was quicker in the atrophic skin changes in comparison with that of the normal skin. The difference was significant. (t 7.616 df - 16 $P < 0.001$)

A similar condition arose in a study with Na^{24} in a corresponding manner in 6 diabetics with atrophic skin changes. The mean k value was 0.073 (T ½ 9 5 min.) in the atrophic skin lesions against 0.047 (T ½ 14.9 min.) in the intact skin. The difference was significant (t 2.635 df 5 $0.05 > P > 0.025$).

In a series of patients with old, established traumatic scars in the lower extremities a comparison was made of the disappearance rate in the scar with that in the adjacent skin by the same method. It is well known that the tissue in an old scar is poor in vessels, and consequently the disappearance rate in the scar should be slower than in the adjacent normal skin.

The series consisted of 21 patients without diabetes, their ages ranging from 19 to 61 years. All the patients were able to state how and when the trauma occurred the appearance of the scar corresponded to their statements. It was chiefly a matter of scars from slashes, bruises and post-operative scars. The ages of the scars varied from 2 to 46 years. All of them were bleached and atrophic. Every patient was examined with I^{131} as well as with Na^{24} (Table 19) As was to be expected, a lower disappearance rate of I^{131} as well as Na^{24} was found in the scars when compared with the adjacent skin. The difference was statistically significant (I^{131} : t = 4 894 df = 20 $P < 0.001$ Na^{24} t = 3 439 df = 20 $P < 0.005$).

Thus old traumatic scars differ from atrophic skin lesions in diabetics in that the disapperance of I^{131} and Na^{24} in the scar is significantly slower and in the atrophic skin lesion significantly quicker than in the adjacent intact skin.

In new traumatic scars there is an abundance of vessels in the granulation tissue. Weiber (1959) revealed that with the isotope technique applied to animals the vascularization of the healing wound progressed successively during the first 5 days and then gradually regressed. About a week later the disappearance rate for the scar approached that of the adjacent skin.

Traumatic scars in diabetics of about the same age as the atrophic skin lesions were also compared. A small series of 5 diabetic patients with relatively newly formed traumatic scars (1 to 2 years old) comparable in age to the atrophic skin changes, were tested with I^{131} in a corresponding manner (Table 20) The disappearance rate in the scar was also lower than in the intact skin. The mean k value in the scar was 0.071 and in the adjacent skin 0.088 If the disappearance rate in these scars was compared with that of the atrophic skin lesions (Table 18) the mean k-value 0.071 and 0.106 respectively the difference was significant. (t 2.657 df = 20 $0.02 > P > 0.01$)

As regards the disappearance of I^{131} traumatic scars in diabetics differ from atrophic skin changes of about the same age. This suggests that the atrophic skin lesions are not traumatic but spontaneous. Since the comparison of the ages

TABLE 17 *Disappearance rate of intradermally injected ¹³¹I in two adjacent deposits in the normal skin of the leg*

				Proximal		Distal	
				T ½	k-value	T ½	k value
1	K S	17 yr	female.	6 1	0 114	5 5	0 126
2.	B. G	18 yr	male.	6 0	0.116	6.6	0.105
3	L. A.	19 yr	male.	9 2	0 075	10 5	0.066
4	H A.	27 yr	male.	8.6	0 081	9.0	0.077
5	A. J	28 yr	female.	5.0	0.139	5.8	0.119
6	K. H	35 yr	female.	5 2	0 133	6.2	0.112
7	G S	38 yr	male.	4 3	0 161	10.6	0 065
8	L. B	38 yr	female.	8 2	0 085	8.7	0 080
9	G S	49 yr	male.	6.6	0 105	6.0	0 116
10.	I S	49 yr	female	5 3	0.131	5 3	0 131
11	A. A.	52 yr	female.	12 1	0.057	8 5	0 082
12.	F G	50 yr	male	10 9	0.064	10 1	0.069
13	V L.	56 yr	male.	6.6	0.105	8 4	0.083
14	O L.	59 yr	male.	6 0	0 116	5.6	0 124
15	C. G	59 yr	male.	9.8	0.071	8.6	0.081
			Mean	7.3	0 104	7.7	0.096

) Diabetics with duration of diabetes 5 and 2 years respecti ely

TABLE 18. *Disappearance rate of intradermally injected ¹³¹I in atrophic skin lesions and in adjacent intact skin in the legs of diabetics*

					Intact skin		Atrophic skin lesion	
					T ½	k value	T ½	k-value
1	H. V	17 yr	Dur 11 yr	male	13.0	0.053	5 3	0 131
2	H. J	28 yr	Dur 14 yr	male	7.0	0 099	5 3	0 131
3	B V	33 yr	Dur 15 yr	male	15 5	0 046	8.7	0.080
4	K. A.	35 yr	Dur 20 yr	male.	8 2	0 085	6.0	0 116
5	R. K.	41 yr	Dur 25 yr	female.	6.2	0.112	4 8	0 144
6	M. P	42 yr	Dur 15 yr	male.	10 2	0.068	7.6	0.091
7	I J	44 yr	Dur 22 yr	male	8 5	0.082	8 5	0.082
8	Y G	44 yr	Dur 17 yr	male	7.7	0 090	4.7	0.147
9	A. S	50 yr	Dur 5 yr	male	13 5	0.051	9.0	0.077
10.	E. N	53 yr	Dur 32 yr	male.	10.5	0 066	5.0	0.139
11	R. M.	57 yr	Dur 11 yr	male.	12.9	0.054	6.6	0.105
12	E. T	60 yr	Dur 13 yr	female	8.2	0.085	6 3	0 110
13	M. J	60 yr	Dur 10 yr	male	9 0	0.077	6.8	0.102
14	P B	68 yr	Dur 12 yr	male	9.2	0.071	5 0	0.139
15	R. H.	69 yr	Dur 17 yr	male.	14 5	0 048	10.9	0 064
16	O O	74 yr	Dur 1 yr	male	12.5	0.055	9 5	0.073
17	F F	74 yr	Dur 9 yr	male	13.0	0 053	9.0	0 077
				Mean	10.6	0.071	7 0	0.106

TABLE 20. *Disappearance rate of intradermally injected I^{131} in traumatic scars 1 to 2 years old and in the adjacent intact skin in the leg of diabetics*

	Traumatic scar		Normal skin		
	T ½	k-value	T ½	k-value	Trauma
1. E. E. 38 y. Dur. 14 yr female.	14.5	0.048	7.0	0.099	Traum. scar 1 yr
2. E. M. 26 yr. Dur 8 y male.	9.0	0.077	10.6	0.065	Op scar 2 yr
3. B. H. 45 yr Dur 15 y female.	8.3	0.084	8.2	0.085	Op. scar 1 yr
4. K. B. 60 yr Dur 23 yr male.	9.1	0.076	5.7	0.122	Op. scar 2 yr
5. H. R. 72 yr Dur 8 yr male.	10.0	0.069	9.7	0.071	Traum. scar 1 yr.
Mean	10.2	0.071	8.2	0.088	

*) N ... — clearance.

of the traumatic scars and the atrophic skin changes are subject to a certain amount of uncertainty the Author considers this condition scarcely conclusive.

In order to ascertain if there was any difference in the disappearance rate of I^{131} between intact diabetic and nondiabetic skin the values for the disappearance rate from the intact skin of the 21 patients with traumatic scars (Table 19) were compared with the values from the intact skin of the 17 patients with atrophic skin lesions (Table 18). In both series the patients were comparable as regards age. The mean k value for the non-diabetic skin was 0.105 and for diabetic skin 0.071. The difference was significant (t 3.141 df = 36 $0.005 > P > 0.001$).

Thus the disappearance for I^{131} is slower in diabetic skin than in non-diabetic skin. As previously mentioned there was no difference in the disappearance rate in relation to diabetic duration.

TABLE 19 *D appearance rate of I^{131} and Na^{24} in old traumatic scars and in adjacent normal skin in the legs of non-diabetics*

	I^{131} Normal skin T ½	I^{131} Normal skin k value	I^{131} Traumatic scar T ½	I^{131} Traumatic scar k-value	Na^{24} Normal skin T ½	Na^{24} Normal skin k-value	Na^{24} Traumatic scar T ½	Na^{24} Traumatic scar k value	Trauma
1 I S 19 yr male	8.0	0.087	10.8	0.064	8.2	0.085	17.1	0.041	Op. scar 3 yr
2 H B. 20 yr male	5.8	0.119	7.0	0.099	4.3	0.161	6.9	0.100	Traum. scar 8 yr
3 L. K. 22 yr male	9.2	0.075	8.4	0.083	9.9	0.070	11.2	0.062	Traum. scar 2 yr
4 A. W 25 yr male	6.7	0.103	7.7	0.090	6.4	0.108	7.9	0.088	Op. scar 10 yr
5 L T 26 yr male	3.5	0.198	10.7	0.065	8.5	0.082	11.8	0.059	Traum. scar 15 yr
6. J F 30 yr male	7.0	0.099	9.8	0.071	7.6	0.091	7.8	0.089	Traum. scar 18 yr
7 F W 31 yr male	4.8	0.144	8.3	0.084	6.4	0.108	7.0	0.099	Traum. scar 16 yr
8 K J 32 yr male	8.0	0.087	16.7	0.042	11.4	0.061	27.3	0.025	Burn. scar 22 yr
9 H. S 34 yr male	5.3	0.131	11.4	0.061	7.6	0.091	9.9	0.070	Traum. scar 24 yr
10. K. M. 42 yr male	5.0	0.139	8.4	0.083	5.8	0.119	8.0	0.087	Traum. scar 30 yr
11 S J 45 yr male	8.8	0.079	10.2	0.068	9.7	0.071	11.8	0.059	Traum. scar 29 yr
12. H M. 48 yr male	10.0	0.069	16.0	0.043	9.9	0.070	18.2	0.038	Traum. scar 27 yr
13 S. J 49 yr male	5.5	0.126	7.7	0.090	4.9	0.141	9.1	0.076	Traum. scar 35 yr
14 H. L 52 yr male	8.9	0.078	13.9	0.050	8.0	0.087	11.8	0.059	Op. scar 24 yr
15 H. L. 52 yr male	8.9	0.078	10.0	0.069	7.1	0.098	6.1	0.114	Traum. scar 35 yr
16. H O 55 yr male	8.7	0.080	9.2	0.075	5.7	0.122	12.5	0.055	Traum. scar 18 yr
17 B. K. 56 yr male	4.1	0.169	4.9	0.141	8.2	0.085	5.1	0.136	Traum. scar 46 yr
18 B H. 56 yr male	7.2	0.096	12.0	0.058	8.6	0.081	10.3	0.067	Op scar 3 yr
19 H. W 59 yr male	7.9	0.088	9.6	0.072	13.5	0.051	16.5	0.042	Op. scar 11 yr
20. M L 61 yr male	10.5	0.066	10.3	0.067	13.3	0.052	17.1	0.041	Traum. scar 30 yr
21 T J 63 yr male	7.2	0.096	17.5	0.040	9.8	0.071	9.5	0.073	Traum. scar 45 yr
Mean	7.2	0.105	10.5	0.072	8.3	0.091	11.6	0.071	

2. Two patients with systemic lupus erythematosus (SLE) were also examined. Both of them had atrophic skin lesions of the same appearance as those seen in diabetics. One of them had also diabetes of one year's duration. In one of these patients the specimens were taken immediately after death.

3. As a control normal skin from the lower extremities was examined in two healthy persons without diabetes or cardiovascular disease.

RESULTS

1. In the controls a very weak, diffuse fluorescence was observed in the epithelial cells of the epidermis. The specificity test revealed no significant decrease and the finding was considered unspecific. Otherwise no certain fluorescence was seen.

2. In the non-controls three different types of fluorescence were observed

a. Fluorescence of the epithelial cells in the epidermis. This was seen partly as a diffuse yellowish-green colour similar to that in the normal cases, and partly as a pronounced fluorescence around the cells. The latter fluorescence was generally more distinct in the basal cell layer of the epidermis. It was observed in the specimens from the 2 patients with SLE and in 4 of the diabetics. It diminished but was not completely eliminated at the control tests and was considered probably specific. (Fig. I—III p. 74). It was not observed in the controls.

b. The specimens from all the diabetics and the two patients with SLE showed a diffuse partly speckled but rather weak fluorescence of the dermis immediately under the basal cell layer of the epidermis. The fluorescence was entirely inhibited at the control tests. It could not with certainty be correlated to PAS-positive structures.

These two types of fluorescence were not only observed in the atrophic skin lesions, but also in the normal adjacent skin. They were considered probably specific. Corresponding fluorescence was not found in the controls.

c. Fluorescence of small vessels in the dermis immediately under the basal cell layer of the epidermis. This fluorescence was entirely eliminated at the control test and could definitely be correlated to thickened, strongly PAS-positive capillary walls. (Fig. IV p. 75). This fluorescence was considered specific. It was observed in the specimens from the two patients with SLE and all the 6 patients with diabetes. It was seen in the atrophic skin lesions as well as in adjacent normal skin from the two diabetic patients examined. It was not seen in the controls.

DISCUSSION

The investigation appears to speak in favour of the fact that bound gammaglobulin may occur in the thickened PAS-positive capillary walls in the intact skin and in the atrophic skin lesions on the lower extremities of diabetics. The condition was analogous in two cases with SLE. Burnham et al (1963) and Cormane (1964) report the incidence of »bound» gammaglobulin in the basal membranes of the epidermis in skin lesions from inter alia discoid lupus and systemic lupus erythematosus. The fluo-

CHAPTER IX

AN INVESTIGATION WITH THE FLUORESCENT ANTIBODY TECHNIQUE ON THE OCCURRENCE OF GAMMAGLOBULIN IN SKIN FROM THE LOWER EXTREMITIES OF DIABETICS

By

Olle Larsson & Hans Melin

It has previously been established that the PAS-positive glomerular and vascular changes in diabetic nephropathy contain gammaglobulin (Odin and Törnblom 1959 Freedman et al 1960) and probably complement (Freedman and Markowitz 1962)

It was thought therefore that it would be of interest to investigate the atrophic skin lesions on the lower extremities in diabetics (Melin 1962 1964) with *the fluorescent antibody technique to* establish the presence of bound gammaglobulin In these skin lesions as well as in the intact skin of diabetics PAS-positive vascular changes have also been observed.

METHOD

Skin was taken with punch biopsy from the lower extremities. The specimens were immediately frozen and subsequently cut into 5 micron thick sections in cryostate and fixed in cold acetone after air-drying

The fluorescent staining was carried out according to Coon's direct method (cf Mellors 1959) *The specimens were* stained with fluorescent rabbit antihuman gammaglobulin serum for 30 minutes at room temperature The specificity of the staining was checked partly by some specimens being pre-treated for 15 minutes with non-conjungated antihuman gammaglobulin serum and partly by some specimens being exposed to gammaglobulin and fluorescent antihuman gammaglobulin serum at the same time.

Only if an obvious diminishing of the fluorescence could be registrated was the result assessed specific.

*Rabbit antihuman gammaglobulin se*rum conjugated with fluorescein isothiocyanate absorbed once with monkey kidney and twice with calf liver was used (Serum FS 365 received by Professor Astrid Fagraeus-Wallbom Statens Bakteriologiska Laboratorium Stockholm Sweden)

At least 4 specimens from each biopsy were stained, and equally as many for control In 2 cases PAS-staining was performed on every other section of the skin specimen

PATIENT MATERIAL

1 Six diabetics with atrophic skin lesions on the lower extremities were examined. In 2 of them the specimens were also taken from clinically normal skin a few cm from the lesions.

CHAPTER X

DISCUSSION

The described atrophic skin lesions in diabetics are, according to the foregoing presentation, almost completely found in the lower extremities. They occur preferably only in diabetics. The group of patients with these skin lesions and without glucosuria or an earlier known diabetes, revealed, according to the oral glucose tolerance test, a change of the glucose metabolism in a diabetic direction. In patients with juvenile onset diabetes these skin lesions were observed only in those with a relatively long diabetic duration. In patients with maturity onset diabetes the lesions occurred after a shorter diabetic duration. The patients with atrophic skin lesions but without glucosuria were all middle aged or elderly people. The connection between the occurrence of the atrophic skin lesions and the incidence of so-called long-term diabetic complications of the vascular type was pronounced.

The aforementioned facts and the trophic nature of the lesions led to the presumption that they were of vascular origin. Thus the subsequent investigation concentrated upon the search for a local arterial occlusion being the cause of the lesions.

The microangiographic investigation revealed no local occlusion. Instead it showed that the dermis below the lesions usually displayed an increased vascularization when compared with the adjacent normal skin. The established increase of the disappearance rate of a locally injected isotope (I^{131} and Na^{24}) in the atrophic skin lesions compared to the nearby intact skin suggests an increased local circulation in the lesions.

There is naturally the possibility of the atrophic lesions having a traumatic origin. Localization in the lower parts of the legs and the dominant occurrence in males may support such an assumption. It may, however, be pointed out that only a fourth of the males with atrophic skin lesions had heavy manual work. A third had light work such as sedentary duties and so on. In the group of active football players without diabetes none of them had corresponding atrophic skin lesions.

A comparison of the disappearance of an isotope (I^{131}) injected into these skin changes with the disappearance in proved traumatic scars on the lower legs from non-diabetic patients, showed that the isotope vanished considerably slower from the traumatic scars. This observation does not justify any definite conclusion being drawn in view of the origin of the atrophic lesions. The traumatic scars were older than the atrophic skin lesions.

The atrophic skin lesions were also different as regards the disappearance of

rescent antibody technique was also used here. No specific fluorescence was observed by these authors in the skin from normal persons. Cormane (1964) also observed a corresponding but weaker fluorescence in apparent normal skin in a few cases with SLE. This fluorescence was similar to that found in this investigation in the basal cell layer of the epidermis in diabetics (see 2 a.)

Specific fluorescence in the small vessels in the skin correlated to thickened PAS-positive capillaries does not appear to have been described previously either in SLE, diabetes or other disorders.

CONCLUSION

With the aid of the fluorescent antibody technique for the demonstration of human gammaglobulin in the skin from diabetics with atrophic skin changes the Authors have been able to demonstrate specific fluorescence of the thickened PAS-positive walls of the vessels in the dermis immediately under the *basal cell layer of the epidermis.*

In 4 of 6 diabetic patients fluorescence was also observed around the cells *in the basal cell layer of the epidermis.* This was considered probably specific. The latter fluorescence has certain similarities with that observed by other investigators in inter alia discoid lupus and SLE. It was not only observed in the atrophic skin lesions on the lower extremities, but also in the apparently normal skin from diabetics. The fluorescence in SLE has similarly been described not only in skin lesions, but also in clinically intact skin. Corresponding fluorescence was not observed in the examination of skin from normal patients.

REFERENCES

Burnham, T. K., Neblett, T. R. & Fine, G.: J. Invest. Derm. *41*: 451. 1963.

Cormane, R. H.: Lancet. *1*: 534. 1964.

Freedman, P., Peters, J. M. & Kark, R. M.: Arch. ntern. Med. *105*: 524. 1960.

Freedman, P. & Markowitz, S.: J. Clin. Invest. *41*: 328. 1962.

Melin, H.: Nord. Med. 67: 661. 1962.

Melin, H.: Acta med. scand. Suppl. 423. 1964.

Mellors, R. C.: Analytical Cytology. 2nd ed. New York, McGraw-Hill Book Company Inc., 1959.

Müller-Eberhard, H. J., Odin, L. & Törnblom, N.: Nord. Med. *61*: 813. 1959.

Odin, L. & Törnblom, N.: Acta Soc. Med. upsalien. *64*: 313. 1959.

CHAPTER X

DISCUSSION

The described atrophic skin lesions in diabetics are, according to the foregoing presentation, almost completely found in the lower extremities. They occur preferably only in diabetics. The group of patients with these skin lesions and without glucosuria, or an earlier known diabetes, revealed, according to the oral glucose tolerance test, a change of the glucose metabolism in a diabetic direction. In patients with juvenile onset diabetes these skin lesions were observed only in those with a relatively long diabetic duration. In patients with maturity onset diabetes the lesions occurred after a shorter diabetic duration. The patients with atrophic skin lesions but without glucosuria were all middle aged or elderly people. The connection between the occurrence of the atrophic skin lesions and the incidence of so-called long-term diabetic complications of the vascular type was pronounced.

The aforementioned facts and the atrophic nature of the lesions led to the presumption that they were of vascular origin. Thus the subsequent investigation concentrated upon the search for a local arterial occlusion being the cause of the lesions.

The microangiographic investigation revealed no local occlusion. Instead it showed that the dermis below the lesions usually displayed an increased vascularization when compared with the adjacent normal skin. The established increase of the disappearance rate of a locally injected isotope (I^{131} and Na^{24}) in the atrophic skin lesions compared to the nearby intact skin suggests an increased local circulation in the lesions.

There is naturally the possibility of the atrophic lesions having a traumatic origin. Localization in the lower parts of the legs and the dominant occurrence in males may support such an assumption. It may however be pointed out that only a fourth of the males with atrophic skin lesions had heavy manual work. A third had light work such as sedentary duties and so on. In the group of active football players without diabetes none of them had corresponding atrophic skin lesions.

A comparison of the disappearance of an isotope (I^{131}) injected into these skin changes with the disappearance in proved traumatic scars on the lower legs from non-diabetic patients, showed that the isotope vanished considerably slower from the traumatic scars. This observation does not justify any definite conclusion being drawn in view of the origin of the atrophic lesions. The traumatic scars were older than the atrophic skin lesions.

The atrophic skin lesions were also different as regards the disappearance of

I^{131} from proved traumatic scars of about the same age in diabetics. The disappearance was significantly slower in the traumatic scars It should however be noted that there is some uncertainity as regards the congruity in age between the atrophic skin lesions and the traumatic scars Conformity between the two compared groups of diabetic patients concerning age at the onset of diabetes and diabetic duration has not been proved either Furthermore, it is possible that a difference concerning the type of probable trauma may be of importance. The traumatic scars were inter alia caused by operative measures.

The fact that the disappearance of a locally injected isotope is slower even in these traumatic scars in diabetics than in the atrophic skin lesions does not in the opinion of the Author support the notion that the lesions are of traumatic origin but that they occur spontaneously

In one case it was possible to assert definitely that the atrophic skin lesions had occurred spontaneously The primary lesions were of a haemorrhagic nature and were neither tender nor irritable They subsequently developed into typical atrophic skin lesions

With regard to the absence of local clinical signs of infection and the histological picture it is improbable that the atrophic skin changes are caused by a local infection Among the patients with juvenile diabetes it was seen that these changes only occurred in those who had had diabetes for a relatively long time — a fact that also excludes an infectious origin

At the histological examination of the skin of diabetics with atrophic skin lesions it was observed that there were changes of the small vessels both in areas corresponding to the skin lesions and in the adjacent skin of the type described by Goldenberg et al (1959)

A comparison of the disappearance of I^{131} in clinically normal skin on the legs of diabetics with the disappearance of I^{131} in corresponding skin areas of non diabetics showed that the disappearance was slower in diabetics. It is possible that there is some connection between the two described findings. Diabetic skin with changes of the small vessels and with a reduced circulation may be more vulnerable than normal skin It is also possible that diabetic skin reacts in another way than normal skin to a trauma. The fact that an isotope dissappears quicker from the atrophic skin lesions than from the aforementioned traumatic scars in diabetics with those reservations already made does not suggest that such is the case

At the examination of the skin from diabetics with atrophic skin lesions with Coon's immunofluorescent technique concerning the occurrence of gammaglobulin fluorescence was observed partly in the capillary walls immediately below the epidermis and partly in the basal cell layer of the latter The latter fluorescence was similar to that which authors have described in discoid and systemic lupus erythematosus. These fluorescence phenomena were observed not only in the atrophic skin changes, but also in the apparently normal skin of diabetics. Fluorescence in systematic lupus erythematosus has also been described not only in obviously changed skin areas, but also in normal skin Corresponding fluores-

cence was not observed in the skin from the controls.

Organ involvements in systemic lupus erythematosus are generally considered to be due to immunological changes. The presence of gammaglobulin in the skin of diabetics with a localization similar to that in systemic lupus erythematosus warrants the presumption that the atrophic skin lesions are due to immunological changes. However this question must remain unanswered.

The basis for the assumption that the atrophic skin lesions are spontaneous and possibly conditioned by immunological changes has already been mentioned. The fact that they are nearly always found in the lower extremities obviously does not suggest a spontaneous origin. In this connection it should be pointed out that a non-traumatic lesion such as erythema nodosum is chiefly localized in the lower extremities.

The more common occurrence of atrophic skin lesions in diabetic males than in diabetic females is difficult to explain. It should, however be borne in mind that e. g. polyarteritis nodosa occurs more frequently in males and lupus erythematosus and erythema nodosum is more common in females.

The atrophic skin lesions in diabetes should be considered a common and typical long-term diabetic complication. They have a characteristic appearance and are easily observed. Owing to their localization they are accessible for further detailed studies.

SUMMARY

A survey is presented of the so-called long term diabetic vascular changes and of skin lesions associated with diabetes. A previously unknown skin lesion in diabetes is described. It consists of small rounded brownish atrophic and circumscribed lesions localized in the lower extremities. They appear isolated, or in groups or are sometimes of a linear pattern. It is shown that these skin changes disappear after one or more years, and that in the meanwhile new ones appear the picture as a whole remaining unchanged. *More often than not the pat*ients are not conscious of the lesions. Some patients consider them to be, in-so-far that they have noticed them, an expression of trauma. On the other hand some patients assert that they arose spontaneously. In one case it was possible to state definitely that the changes had occurred spontaneously.

A diabetic material of 277 patients, all *of them more than 15 years of age* were examined as regards the occurrence of the aforementioned skin lesions and so-called diabetic complications. The skin changes arose in 65 per cent of the males and in 29 per cent of the females. The incidence of the lesions was correlated to the diabetic duration and increased with the latter. *The lesions occurred* earlier in *maturity onset* diabetics than in juvenile onset ones. The atrophic skin lesions were correlated to the incidence of long-term diabetic vascular complications such as retinopathy and nephropathy. The skin lesions were also correlated to neuropathy and to arterial disease of the lower extremities. On the other hand they were not correlated to the incidence of hypertension.

The occurrence of atrophic skin lesions of the described type in non-diabetics is very rare. A material of 104 patients with hypertension comparable in age, but without diagnosed diabetes, were examined to see if the arteriolosclerotic vascular changes in this disease could possibly give rise to such lesions. Only 7 patients had skin lesions similar to those described. The oral glucose tolerance test showed that 6 of these patients had a diabetic tolerance curve.

A group of 17 patients with atrophic skin lesions congruent to those described but without glucosuria or earlier known diabetes were examined with the oral glucose tolerance test. They had a statistically proved change of the glucose tolerance in a diabetic direction as compared to a control material of a similar age.

In 2 patients with *systemic lupus erythematosus skin lesions in the lower* extremities were discovered which were congruent to the diabetic. One of these patients had a diabetic glucose tolerance

curve. In a further 28 patients with severe collagen disease no corresponding skin lesions were found.

At the histological examination of the skin lesions atrophy of the epidermis and sometimes slight fibrosis of the dermis were demonstrated. Pigment-stainings revealed no definite increase of the melanin content, and in a few cases an inconsiderable quantity of hemosiderin was found. Moreover PAS-positive vascular changes of the type previously described by other authors were observed, partly in the skin lesions and partly in the apparently intact skin.

A microangiographic study of autopsy material from diabetics with atrophic skin lesions showed that the dermis corresponding to the changes was often more abundantly vascularized than the adjacent intact skin.

Disappearance studies with a locally injected isotope (I^{131} and Na^{24}) showed that there was a significantly quicker disappearance of the isotope in the atrophic skin lesions than in the nearby intact skin. In proven traumatic scars on the lower extremities of non-diabetics the disappearance was slower in the scars than in the neighbouring skin I diabetics with traumatic scars of about the same age as the skin lesions the disappearance of I^{131} was also slower in the scars than in the adjacent skin. The difference in the disappearance rate between the latter scars and the skin lesions was statistically significant. The importance of these observations is discussed. They are considered to speak in favour of the atrophic skin lesions not being of traumatic origin and that they occur spontaneously.

A fluorescence-microscopic examination of skin from diabetics with atrophic lesions with anti-human gammaglobulin revealed fluorescence partly in the capillary walls immediately under the epidermis, and partly in the basal cell layer of the epidermis. The latter fluorescence was similar to that demonstrated by former investigators in skin from patients with discoid and systemic lupus erythematosus. As in systemic lupus erythematosus this fluorescence was not only observed in the skin lesions, but also in apparently intact skin. The possibility of the atrophic skin lesions in diabetes being due to changed immunologic conditions is discussed.

The atrophic skin lesions in diabetes are a common and long-term diabetic complication which, as regards their site are easily accessible for further detailed studies.

T all those who ha in various ways contributed to the completion of this investigation I take this opportunity of expressing my thanks. I am very much indebted to Professor Lars-Gunnar Larsson for his valuable help and for permission to use the isotope equipment in his department, and to Mr Lars Jonsson for his generous aid in electro-technical matters. T Professor Carl-Martin Fasey I am deeply indebted for his guidance and constructive criticism the histological examination. Thanks are also due to Professor Stig Borgstrom for kindly

placing the micro-angiographic apparatus at my disposal and to Docent Bo Odén whose skilful aid enabled me to become conversant with the micro-angiographic technique. I am very much obliged to Professor Einar Hollström for his good advice and criticism concerning dermatological problems. Thanks are also due to Mrs Margit Leonardsson for her indefatigable help with the patients. To the Staffs of the Departments of Ophthalmology and Clinical Chemistry I would like to express my thanks for their valuable assistance. Docents Gunnar Borg and Wilhelm van der Linden generously gave me statistic advice. Valuable help was received from Farbenwerke Hoechst, Frankfurt. The Investigation was supported by grants from the Medical Faculty of the Umeå University, Nordisk Insulinfond, Copenhagen and Längmanska kulturfonden, Stockholm

REFERENCES

Agersen, O & Moe, H Diabetes 10: 253 1961.

Agersen, O Neurovascular Examinations on the Lower Extremities in Young Diabetics, with Special Reference to the Autonomic Neuropathy Copenhagen, C. Hamburgers Bogtrykkeri 1962.

Angervall, L., Dorevall, G. & Lehman, K.-E Acta med. scand. 169 339 1961

Angevine, M D The P thogenesis of Polyarteritis. In Mills, L C & Moyer J H. ed.: Inflammation and Disease of Connective Tissue Philadelphia, W B Saunders, 1961

Ashton, N Brit. J Ophthal 33 407 1949

Ashton, N Brit. J Ophthal 35 189 1951

Ballantyne, A. J & Loewenstein, A T Ophthal. Soc U K. 63 95 1943

Ballantyne A J & Loewenstein, A. Brit J Ophthal. 28 593 1944

Ballantyne, A J Arch. Ophthal 33 97 1945

Bärány F R Acta med scand suppl. 304 1955

Barron, J N Veall, N & Arnott, D C Brit. J. plast. Surg 4 16, 1951

Bauer F K Camera, B Yourtcheff L & Shoop, L Amer J med Sci. 225 374 1953

Bech, K Hansen, L Lorentzon, S L & Lundbaek, K Diabetes 9 441 1960.

Bell, E T Arch. Path. 49 469 1950.

Bell, E T Arch Path 53 444 1952

Bell, E T Diabetes 2 376, 1953

Bell, E T Amer J clin P th 28 27 1957

Bellman, S Acta radiol suppl 102, 1953

Bergstrand, A & Bucht, H Lab Invest. 6. 293 1957

Bettley F R. & Fairburn, E. A Acta derm venereol. Proc. 11th Internat Congress Derm 601 1957

Bischoff, A. Schweiz. med. Wschr 89 519 1959

Blair D A Glover W E & Roddie, I. C. J Physiol. (Lond.) 149 19 P 1959

Blair D A, Glover W E & Roddie, I C. J Physiol. (Lond) 153 232, 1960.

Blocker T G. Jr Lewis, S. R, Perry J L, Trombeck, W T & Lynn, W L Ann. Surg. 145 630, 1957

Blumenthal, H T Alex, M. & Goldenberg, S.. Arch. P th. 70 13 1960.

Bradley R. F & Bryfolge, J W.: Amer J Med. 20 207 1956.

Braithwaite, F Farmer F T & Herbert, F L Brit. J plast. Surg. 4 38, 1951

Bryfolge, J. W & Bradley R. F Diabetes 6 159 1957

Chesrow E J & Bleyer J M. Geriatrics 9 276, 1954

Chesrow E J & Bleyer J M. Geriatrics 12. 171 1957

Clawson, B. J & Bell, E. T Arch. P th. 48 105 1949

Conn, J W.: Amer J med Sci. 199 555 1940.

Conn, J W & Arbor A. Diabetes 7 347 1958

Crombie, D L. & FitzGerald, M. G Brit med J 1497 1962.

Dahlberg, G Jorpes, E Kallner S. & Lichtenstein, A. Acta med. scand. suppl. 188, 1947

Dayton, A., Dobson, H. L & Brennan, J C.: Ann. intern. Med. 54 672, 1961

Ditzel, J Circulation 10 277 1954

Ditzel, J The Conjunctival Vessels in Diabetes Mellitus. Copenhagen, Munksgaard, 1962.

Dodd, H. & Cockett, F B The P thology and Surgery of the Veins of the Lower Limb. London, Livingstone, 1956.

Dolger, H J Amer med. Ass. 134 1298, 1947

Dry T J. & Hines, E. A. J Ann. intern. Med. 14 1893, 1941

placing the micro-angiographic apparatus at my disposal, and to Docent Bo Odén whose skilful aid enabled me to become conversant with the micro-angiographic technique. I am very much obliged to Professor Einar Hollström for his good advice and criticism concerning dermatological problems. Thanks are also due to Mrs Margit Leonardsson for her indefatigable help with the patients. To the Staffs of the Departments of Ophthalmology and Clinical Chemistry I would like to express my thanks for their valuable assistance. Docents Gunnar Borg and Wilhelm van der Linden generously gave me statistic advice. Valuable help was received from Farbenwerke Hoechst, Frankfurt. The investigation was supported by grants from the Medical Faculty of the Umeå University, Nordisk Insulinfond, Copenhagen and Längmanska kulturfonden, Stockholm.

McGirr E M Clin. Sci. *11* 91 1952.

MacKenzie Royal London Ophthalmic Hosp. Report *9* 352, 1877 Cit. in Joslin, E P Root, H. F White, P & Marble, A. ed The Treatment of Diabetes Mellitus. 10th ed Philadelphia, Lea & Febiger 1959

McManus, J. F A Amer. J Path. *24* 643 1948

Megibow R S Megibow S J Pollack, H Bookman, J J & Ostermann, K. Amer J Med. *15* 322, 1953

Mellors, R. C Analytical Cytology 2nd ed New York. Mc Graw-Hill Book Comp 1959

Melin, H Nord. Med. *67* 661 1962

Mendlowitz, M Grossman, E B & Alpert, S Amer J Med. *15* 316 1953

Montagna, W The Structure and Function of Skin 2nd ed. New York, Academic Press, 1962.

Mowenthal, H O & Berry E Ann intern Med. *33* 1175 1950.

Mårtensson, J Acta med scand. *138* 94, 1950

Nettleship Trans. Ophthal Soc United Kingdom *8* 159 1888 Cit. in Joslin, E P Root, H F White, P & Marble, A ed The Treatment of Diabetes Mellitus 10th ed Philadelphia, Lea & Febiger 1959

Oakley W Catterall, R C F & Martin, M. M Brit med J 22 953 1956

Oberdisse, K Blank, H. & Huter K A Klin Wschr 40 446, 1962

Odeblad, E & Westin, B Acta radiol suppl 173 1959

Odén, B Bellman, S & Fries, B Br J Radiol *31* 70, 1958

Odin, L & Torablom, N Acta Soc Med upsalien 64 313 1959

Oppenheim, M Zbl Haut-u Geschl Kr *32* 179 1929

Pabst, H W Uber die Anwendung von radioaktivem Jod zur Untersuchung der periferen Durchblutung und ihrer pharmakologischen Beeinflussung In Fellinger K. & Vetter H ed Radioaktive Isotope in Klinik und Forschung Bd 35 Munchen-Berlin, Urban & Schwarzenberg, 1955

Pedersen, J & Olsen, S Acta med. scand *171* 551 1962.

Pillsbury D M., Shelley W B & Kligman, A. M. Dermatology Philadelphia, W B. Saunders Comp., 1957

Philipp, K. & Rauf F Strahlentherapie 90 553 1953

Prud'Homme, J Un. méd. Can. *89* 616 1960.

Pyke, D A & Roberts, D S. Cit Acta med. scand *163* 489 1959

Rapaport, S. I., Saul, A., Hyman, C. & Morton, M E Circulation *5* 594 1952.

Ries, W Schuster L., Serge, K. & Wegner H Klin Wschr *38* 681 1960.

Rinehart, J F & Abul-Haj, S. K. Arch. Path. *52* 189 1951

Rodriguez, R & Root, H. F New Engl. J Med. 238 391 1948.

Rollins, T G & Winkelmann, R. K. Arch Derm. *82* 537 1960.

Root, H F The Nervous System and Diabetes In Joslin, E P Root, H F White, P & Marble A ed The Treatment of Diabetes Mellitus. 10th ed Philadelphia, Lea & Febiger 1959

Rotwit, B Washam, L H. & Sorrentino, J Amer J Roentgenol. *69* 980, 1953

Rundles, R W Medicine (Baltimore) *24* 111 1945

Sabour M S McDonald, M. K. & Robson, J S Diabetes *11* 291 1962

Salus, R Med. Klinik *1* 256 1928

Scott, A Trans. St John Hosp. derm. Soc. (Lond) *46* 22, 1961

Segroth, K Acta med. scand suppl 325 1957

Spalteholz, W Blutgefässe der Haut. In Jadassohn, J. ed. Handbuch der Haut- und Geschlechtskrankheiten. Erster Band. Erster Teil. Berlin, J. Springer 1927

Sonnesson, I Acta med scand. suppl. 394, 1963

Stone, P W & Miller W B Proc. Soc. exp. Biol (N Y) *71* 529 1949

Sutton, R. L Diseases of the Skin. 11th ed. S Louis, C V Mosby Comp 1956.

Teller J D Abstr of Papers. 130th Meeting. Amer Chem. Soc. 69 C, 1956

Urbach, E. Arch. Derm. Syph. (Berl) *166*, 273 1932.

Urbach, E J Amer med Ass. *129* 439 1945

Walder D N Clin. Sci *12* 153 1953.

Elkin D C, Cooper F W Rohrer R H, Miller W B Shea P C & Dennis E W: Surg. Gynec. Obstet. 87 1 1948.

Fagerberg, S E. Acta med. scand suppl. 345 1959

Fajans, S S & Conn J W Diabetes 3 296 1954

Farquhar M G Hopper J & Moon H D Amer J Path. 35 721 959

Fitzpatrick, T B Dermatologic Lesions and Diseases associated with Diabetes. In Williams, R. H ed Diabetes. New York, P B Hoeber 1960.

Foley W T & Wright I S Color Atlas and Management of Vascular Disease. New York, Appleton-Century-Crofts, 1959

Goldenberg, S Alex M. & Blumenthal H. T: Diabetes 7 98, 1958

Goldenberg, S Alex, M., Joshi R. A. & Blumenthal, H T: Diabetes 8 261 1959

Goodman J I Baumoel S Frank I L., Marcus, L. J & Wassermann, S: The Diabetic Neuropathies. Springfield Illinois C. C. Thomas, 1953

Goodof I I Ann intern. Med 22 373 1945

Guilford J P Fundamental Statistics in Psychology and Ed cation 2nd ed New Yo k Mc Graw Hill Book Comp., 1950

Guy W B Penn med. J 54 1052 1951

Cirde, A. & Kugelberg, E. Nord Med. 70 1252 1963

Handelsman M B Morrione T G & Ghiman B: Arch intern. Med 110 108 1962

Hanser W H., Tauxe W N Owen, C. A & Lipscomb P R. Surg. Gynec. Obstet 112 625 1961

Hanum S Acta ophthal. (Kbh) suppl. 16 1939

Hare, P J Brit. J Derm. 67 365 1955

Hecht, A Weisenfeld S & Goldner M. G Metabolism 10 712, 1961

Hollander W Reilly P Burrows B A. J clin. Invest. 40 222 1961

Hyman, C Rapaport S J & P ldno R Amer J Physiol 163 722 1950

Ikkos, D & Luft, R Acta endocr (Kbh) 25 312 1957

Jackson, W I U Brit med J 11 690, 1952

Jordan W Arch. intern Med. 57 307 1936

Joslin, E. P Root, H F White, P & Marble A. The Treatment of Diabetes Mellitus. 10th ed. Philadelphia, Lea & Febiger 1959

Keston, A: Abstr of Papers 129th Meeting Amer Chem. Soc. 31 C, 1956

Kety S S Amer J med. Sci. 215 352, 1948

Kety S. S: Amer Heart J 38 321 1949

Kety S S: Med. Sci. Publ. 3 128 1953

Kimmelstiel P & Wilson, C.: Amer J Path. 12 83 1936

Kurz O Arch Augenheilk. 110: 284 1937

Kutt, H., Hurwitz, L. J: Ginsburg S M. & McDowell, F: Arch Neurol. (Chic.) 4 31 1961

Lax, H. & Feinberg, A. W Circulation 20 1106 1959

Lever W F: Histopathology of the Skin 3rd ed. Philadelphia J B Lippincott Comp., 1961

Levin, K. & Linde, S Svenska Läk.-tidn. 59 3016 1962

Liebow I M Hellerstein, H K. & Miller M. Amer J Med 18 438 1955

Lisa J R., Magiday M., Galloway I & Hart, J F: J Amer med. Ass. 120 192 1942

Locke S Lawrence D G & Legg, M. A. Amer J Med 34 775 1963

Lundbaek, K. Ugeskr Laeg. 111 1349 1949

Lundbaek K. Long-term Diabetes. Copenhagen, Munksgaard, 1953

Lundbaek K Late Development n Long-term D abetic Vascular Disease In ed. Diabetes Mell tus. III Kongress der Internationalen Diabetes Federation, Dusseldorf 1958 Stuttgart, G Thieme, 1959

Lundbaek, K Medicinsk Årsbok 1960—1961 Stockholm, A.B Nordiska Bokhandeln 1960

Mackay I R. & Burnet F M.: Autoimmune Diseases. Springfield Illino s, C C. Thomas, 1963

Marble, A Infections in Diabetes. In Joslin, E P Root, H F White P & Marble A ed. The Treatment of Diabetes Mell tu 10th ed Philadelphia, Lea & Feb ger 1959

Martin, M M Brain 76 594 1953

Master A M. & Lasser R P Blood Pressure Elevation in the Elderly I Brest, A. N & Moyer J H ed.: Hypertension. Recent Ad as es. Philad lphia Lea & Febiger 1961

McGirr E. M. Clin. Sci. *11* 91 1952.
MacKenzie Royal London Ophthalmic Hosp Report *9* 152, 1877 Cit. in Joslin, E. P Root, H. F White, P & Marble, A. ed The Treatment of Diabetes Mellitus. 10th ed. Philadelphia, Lea & Febiger 1959
McManus, J F A Amer J P th *24* 643 1948
Megibow R. S Megibow S. J Pollack, H Bookman, J J & Osserman, K. Amer J Med *15* 322, 1953
Mellors, R. C. Analytical Cytology 2nd ed New York, Mc Gr w-Hill Book Comp 1959
Melin, H. Nord. Med. *67* 661 1962.
Mendlowitz, M Grossman, E B & Alpert, S. Amer J Med *15* 316, 1953
Montagna, W The Structure and Function of Skin. 2nd ed New York, Academic Press, 1962.
Moestchal, H O & Berry E Ann. intern Med *33* 1175 1950
Mikkelsen, J Acta med. scand. *138* 94 1950
Nettleship Trans Ophthal Soc United Kingdom *8* 159 1888. Cit in Joslin, E P Root, H. F White, P & Marble, A ed The Treatment of Diabetes Mellitus 10th ed Philadelphia, Lea & Febiger 1959
Oakley W Catterall, R. C F & Martin, M M Brit med J *22* 953 1956
Oberdisse K Blank, H. & Huter K. A Klin Wschr 40 446, 1962.
Odeblad, E & Westin, B Acta radiol suppl 173, 1959
Odda, B Bellman, S & Fries, B Brit J Radiol *31* 70, 1958
Odin, L & Tornblom, N Acta Soc Med upsalien *64* 313 1959
Oppenheim, M Zbl Haut-u Geschl.-Kr *32* 179 1929
Pabst, H. W Uber die Anwendung von radioaktivem Jod zur Untersuchung der peripheren Durchblutung und ihrer pharmakologischen Beeinflussung I Fellinger K & Vetter H ed Radioaktive Isotope in Klinik und Forschung. Bd 33 Munchen-Berlin, Urban & Schwarzenberg, 1955
Pedersen, J. & Olsen, S Acta med. scand. *171* 551, 1962
Pillsbury D M Shelley W B. & Kligman, A. M. Dermatology Philadelphia, W B Saunders Comp 1957
Philipp, K. & Ruf F Strahlentherapie 90 553 1953
Prud'Homme, J Un. méd. Can. *89* 616 1960.
Pyke, D A & Roberts, D S. C. Acta med. scand. *163* 489 1959
Rapaport, S. L, Saul, A Hyman, C. & Morton, M E. Circulation *5* 594 1952.
Ries, W Schuster L, Seige, K & Wegner H. Klin. Wschr *38* 681 1960
Rinehart, J F & Abul-Haj, S. K. Arch. Path. *52* 189 1951
Rodriguez, R. & Root, H. F New Engl. J Med *238* 391 1948
Rolfins, T G. & Winkelmann, R. K. Arch Derm. *82* 537 1960
Root, H F The Nervous System and Diabetes. In Joslin, E P Root, H. F White, P & Marble A ed The Treatmen of Diabetes Mellitus. 10th ed. Philadelphia Lea & Febiger 1959
Rosew t, B Wuham, L. H. & Sorrentino, J Amer J. Roentgenol. *69* 980, 1953
Ruedles, R. W Medicine (Baltimore) *24* 111 1945
Sabour M S McDonald, M. K. & Robson, J S Diabetes *11* 291 1962
Salus, R Med Klinik *1* 256 1928
Scott, A T ans. St John Hosp derm. Soc. (Lond) *46* 22, 1961
Segroth, K Acta med scand suppl. 325 1957
Spalteholz, W Blutgef sse der Haut In Jadassohn, J ed Handbuch der Haut- und Geschlechtskrankheiten. Erster Band. Erster Teil Berlin, J Springer 1927
Steiness, I Acta med. scand. suppl. 394 1963
Stone, P W & Miller W B Proc. Soc. exp. Biol (N Y) *71* 529 1949
Sutton, R L., Diseases of the Skin. 11th ed. St. Louis, C. V Mosby Comp., 1956.
Teller J D Abstr of Papers. 130th Meeting. Amer Chem Soc. 69 C, 1956.
Urbach, E., Arch. Derm. Syph (Berl.) *166* 273 1932.
Urbach, E J Amer med. Ass. *129* 439 1945
Walder D N. Clin Sc *12* 153, 1953

Elkin D C, Cooper F W Rohrer R H, Miller W B Shea, P C & Dennis, E W: Surg. Gynec. Obstet. 87 1 1948
Fagerberg, S E. Acta med. scand. suppl. 345 1959
Fajans, S S & Conn J W Diabetes 3 296 1954
Farquhar M G Hopper J & Moon, H D Amer J Path. 35 721 1959
Fitzpatrick, T B Dermatolog c Lesions and D seases associated with Diabetes. In Williams, R. H. ed. Diabetes. New York P B Hoeber 1960
Foley W T & Wright I S: Color Atlas and Management of Vascular Disease. New York Appleton-Century Crofts, 1959
Goldenberg, S Alex, M. & Blumenthal, H T: Diabetes 7 98 1958
Goldenberg, S., Alex, M., Joshi, R. A. & Blumenthal H T Diabetes 8 261 1959
Goodman J I Baumoel, S Frankel, L., Marcus, L. J & Wassermann S The Diabetic Neuropathies. Springfield Ill nois, C. C. Thomas, 1953
Goodof I I Ann intern. Med 2 373 1945
Guilford J P Fundamental Statistics in Psychology and Education 2nd ed New York, Mc Graw-Hill Book Comp 1950
Guy W B. Penn med. J 54 1052 1951
Girde A. & Kugelberg, E. Nord. Med. 70 1252 1963
Ha delsman M. B., Morrione T G & Ghitman, B Arch. intern Med. 110 108, 1962
Hanser W H Tauxe, W N Owen C A & Lipscomb P R. Surg Gynec. Obstet 112 625 1961
Han m, S Acta phthal. (Kbh) suppl. 16 1939
Hare, P J Brit. J Derm. 67 365 1955
Hecht, A., Weisenfeld S & Goldner M G Metabolism 10 712 1961
Hollander W Reilly P Burrows, B A J clin Invest. 40 222 1961
Hyman C., Rapaport, S J & Paldino R Amer J Physiol. 163 722, 1950
Ikkos D & Luft, R Acta endocr (Kbh.) 25 312 1957
Jackson W P U Br t. med. J 11 690 1952
Jordan, W Arch ntern. Med. 57 307 1936
Joslin, E. P Root, H F White, P & Marble A: The Treatment of Diabetes Mellitus. 10th ed. Philadelphia, Lea & Febiger 1959
Keston A. Abstr of Papers 129th Meeting Amer Chem. Soc. 31 C, 1956
Kety S. S: Amer J med. Sci. 215 352, 1948
Kety S S Amer Heart J 38 321 1949
Kety S S.: Med. Sci Publ. 3 128 1953
Kimmelstiel, P & Wilson, C. Amer J Path. 12 83 1936
Kurz, O Arch. Augenheilk. 110: 284 1937
Kott, H., Horw tz, L. J Ginsburg, S M. & McDowell F Arch. Neurol. (Chic.) 4 31 1961
Lax, H. & Feinberg, A. W Circulation 20 1106, 1959
Lever W F Histopathology of the Skin 3rd ed Philadelphia J B. Lipp ncott Comp 1961
Levin, K. & Linde, S Svenska Läk.-tidn. 59 3016 1962.
Liebow I. M., Hellerstein, H. K. & Miller M Amer J Med. 18 438 1955
Lisa, J R., Magiday M., Galloway I & Hart J F: J Amer med. Ass. 120 192 1942
Locke, S Lawrence D G & Legg, M A. Amer J Med. 34 775 1963
Lundbaek, K. Ugeskr Laeg 111 1349 1949
Lundbaek, K. Long-term D abetes. Copenhagen Munksgaard, 1953
Lundbaek, K. Late Developement in Long-term Diabetic V scular Disease In ed. Diabetes Mell tus. III Kongress der Internationalen Diabetes Federation, Düsseldorf 1958 Stuttgart, G Thieme, 1959
Lundbaek, K. Medicinsk Årsbok 1960—1961 Stockholm, A.B. Nordiska Bokhandeln 1960.
Mackay I R. & Burnet, F M. Autoimmune Diseases. Springf eld Illino s, C. C. Thomas, 1963
Marbl A. Infections Diabetes. I Joslin, E P Root, H. F Wh te P & Marble, A. ed The Treatment of Diabetes Mellitus. 10th ed. Philadelphia, Lea & Febiger 1959
Martin, M. M. Brain 76 594 1953
Master A. M & Lasser R P Blood Pressure Elevation the Elderly I Brest, A. N & Moyer J H. ed. Hypertension. Recent Advances. Ph ladelphia Le & Febiger 1961



FIG 4 *Microangiogram,* × *125 from normal skin f the lower extremity Autopsy pecimen from non-diabetic patient (E. L. ♀ 32) Even the apillaries in the superficial dermis are visible Micropaque 20 % was used.*



FIG 5 *Microangiogram,* × *125 from an atrophic skin l ion with surrounding clinically intact skin f the lower extremity Autopsy pecimen from diabetic patient (K. A. ♂ 36) The border of the le ion are indicated, Not the abundant f sels with fan-like pread within the patch The surrounding tissue l poorer in visibl ve el The apillaries in the superficial dermis ar hardly seen and the mall ve sels are f irregular lz Micropaque 20 % was us d.*

Warren, S & LeCompte P M. The Pathology of Diabetes Mellitus 3rd ed. Philadelphia, Lea & Febiger *1952*

Welber A. Acta chir scand. suppl. *237* *1959*

Wexler D & Branower G Arch. Ophthal *44* *539* *1950*

Wilson J L., Root, H. F & Marble, A. New Engl. J Med. *245* *513* *1951*

Winter F C.: J Amer med. Ass. *174* 143 1960

Wise, G N Trans. Amer ophthal. Soc. *54* *729* *1956*

Wisham, L. H Yalow R. S & Freund, A. J Amer Heart J *41* 810 1951

Wisham, L. H. & Yalow R. S. Amer Heart J *43* *67* *1952*.

Woltman H. W & Wilder R. M.: Arch. intern Med. *44* *576* *1929*

Zacks, S I Pegues, J J & Elliott, F A. Metabolism *11* 381 1962



FIG 4 Microangiogram, × 12.5 from normal skin of the lower extremity Autopsy pecimen from non-diabetic patient (E L ♀, 32) Even the pillaries in the superficial dermis are isible. Micropaque 20 % was ed



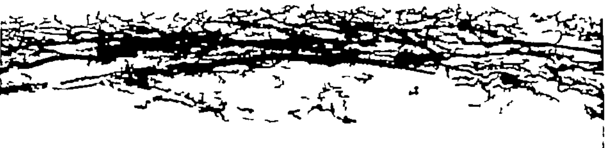


FIG 5 Microangiogram, × 12.5 from an atrophic skin le ion with surrounding linically intact skin of the lower extremity. Autopsy specimen from diabetic patient (K A ♂ 36) The borders f the lesion are indicat d. Not the abundanc of vessel with a fan-like pread within the pat b The urrounding tissue is poorer in isibl ve sels. The capillaries in the superficial dermis are hardly seen and the small ve sel are of irregular siz Micropaque 20 % was ed.



FIG 6 a

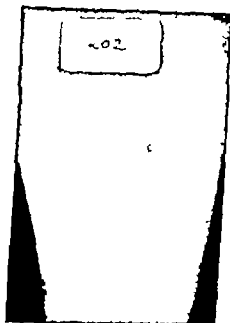


FIG 6 b



FIG 7 a



FIG 7 b

Fig 6 a *J H ♂ (59) Newly discovered diabetes Primary lesion on th left leg*

Fig 6 b *The same lesion fou months later now brown and atrophic*

Fig 7 a *K A ♂ (36) Diabetes 20 year Complications Numerou atrophic skin l sions on both leg*

Fig 7 b *The same patient Left leg at cl er range*

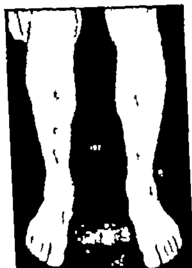


FIG 8



FIG 8 b.



FIG 9

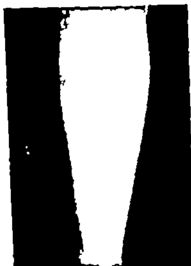


FIG 10

Fig 8 B B ♂ (44) Newly discovered diabetes No complications. High incidence of diabetes in the family Brown atrophic lesions on the leg

Fig 8 b The same patient Left leg at close range

Fig 9 A S ♂ (50) Diabetes in four years No complications. Right leg, lateral and distal The atrophy is clearly visible.

Fig 10 G A. ♀ (29) Diabetes 19 years Complications Brown atrophic lesion on the left leg



FIG 6 a.

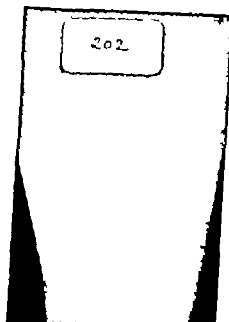


FIG 6 b



FIG 7 a.

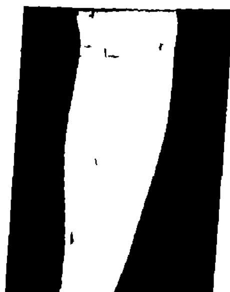


FIG 7 b

F g 6 *J H* ♂ *(59) Newly di covered diabet s Primary l sions on th left leg*

F g 6 b *The same lesions four months later now brown and atrophic*

Fig. 7 a *K A.* ♂ *(36) Diabetes 20 years C mplication Numerou atrophic skin l sions on both leg*

Fig 7 b *The same patient Left l g at loser ange*

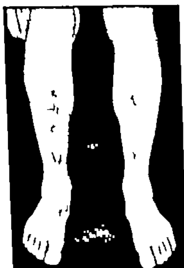


FIG 8



FIG 8b



FIG. 9

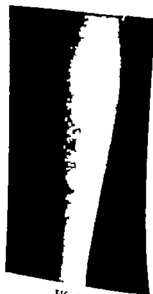


FIG. 10

Fig 8 B. B ♂ (44) Newly discovered diabetes. No complications. High incidence of diabetes in the family. Brown atrophic lesions on the legs

Fig 8b The same patient. Left leg [illegible] close range.

Fig 9 A. S ♂ (50) Diabetes in four years. No complications. Right leg. Lateral and distal. The atrophy is clearly visible

Fig 10 G. A. ♀ (29). Diabetes 19 years. Complications. Brown atrophic lesions on the left leg

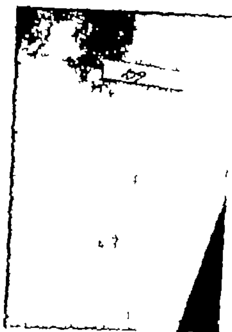


FIG 11



FIG 12



FIG 13 a



FIG 13 b

Fig. 11. V. P. ♂ (34) Newly discovered diabetes. Retinopathy. Left leg lateral. Atrophy and pigmentation clearly seen.

Fig. 12. R. K. ♀ (40) Diabetes 23 years. Neuropathy. Left leg lateral. Linear arrangement of the lesions. Atrophy pronounced in some lesions.

Fig. 13 a. P. B. ♂ (68) Diabetes 11 years. No complications. Note the brown atrophic lesion in the middle of the picture.

Fig. 13 b. The same patient two years later. The enlarged lesions are the same as seen in 13 a. Note the appearance of new lesions.



FIG. 14 a.



FIG 14 b.

Fig. 14 *Y G ♀ (42) Diabetes 15 years. Retinopathy Brown atrophic lesions in linear pattern.*

Fig 14 b *The same patient 16 months later The lesions in 14 have not disappeared and new ones have arisen.*



FIG 15



FIG 16

Fig 15 *E. M ♂ (62) Diabetes 4 years. Complications Autopsy specimen. Atrophic skin l leg PAS-staining × 200. Epidermis is atrophic The capillaries are strongly PAS-positive.*

Fig 16 *N L. ♂ (60) Diabetes 4 years. Retinopathy Autopsy specimen. A marked PAS-positive capillary in the superficial part of the dermis PAS-staining. × 520.*



FIG 11



FIG 12



FIG 13 a



FIG 13 b.

I g 11 *V P ♂ (54) Newly discovered diabete Retinopathy Left leg lateral. At phy and p gmentation clearly seen*

Fig. 12 *R K ♀ (40) Diabet 23 year Neuropathy Left leg lateral Linear arrange ment of the l sion Atrophy is pronounc d in som lesions*

Fig. 13 *P B ♂ (68) Diabetes 11 year No complications N te the brown atrophic le ions in the middle of the pictur*

Fig 13 b *The same patient two years later Th enf amed l ions are the same as seen in 13 a. Note the appearanc f new lesion*



FIG. IV a.

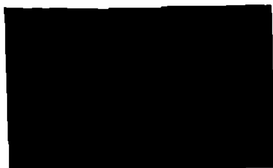


FIG IV b.



FIG IV c.

Fig IV a, IV b and IV c. B. W. ♂ (34) Diabetes 21 years. Complications. Fluorescence micrographs × 320. Skin from the lower leg. Punch biopsy. Epidermis at the top of the pictures. a. and b. Specific fluorescence in the small vessels in the superficial part of the dermis. It corresponded to PAS-positive capillaries. c. Inhibition from the same specimen. Fluorescence of the capillaries is entirely inhibited.

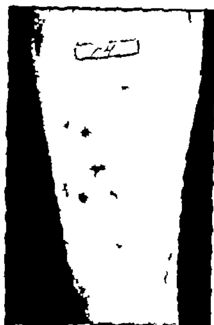


FIG I

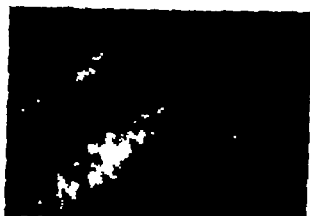


FIG II a.



FIG II b



FIG III

Fig. I *J N ♂ (50) Newly discovered diabetes. No complications Right leg lateral Numerous atrophic brown lesions*

Fig. II a and b *Fluorescence micrographs Atrophic skin lesion from the patient in Fig I Punch biopsy Epidermis at the top of the picture a Specific fluorescence in the b sal layer of epidermis* × *800 b Same specimen* × *320 Inhibition of th specific fluorescence*

Fig. III *L. L. ♀ (50) Systemic l pus erythematosus for 18 years Fluorescence micrograph* × *3·0 Skin from the lower leg Autopsy specimen Epiderm to th ight in the picture Note same fluor scent band in the basal layer of epidermis as in F g II a. Un pecific fluorescen in the horny layer of epiderm*



FIG. IV a.



FIG. IV b



FIG. IV c.

Fig. IV a, IV b and IV c. *B. W. ♂ (34) Diabetes 21 year. Complications. Fluorescence micrograph × 330. Skin from the lower leg. Punch biopsy. Epidermis at the top of the picture a. and b. Specific fluorescence in the small vessels in the superficial part of the dermis. It corresponded to PAS-positive capillaries. Inhibition from the same specimen. Fluorescence of the capillaries is entirely inhibited.*

ACTA MEDICA SCANDINAVICA

SUPPLEMENTUM 422

NET CATION EQUIVALENCY ('BASE BINDING POWER') OF THE PLASMA PROTEINS

A STUDY OF ION-PROTEIN INTERACTION IN HUMAN PLASMA
BY MEANS OF
IN VIVO ULTRAFILTRATION AND EQUILIBRIUM DIALYSIS

by

A. M. VAN LEEUWEN

ACCOMPANIES VOL. 176

ACTA MEDICA
SCANDINAVICA

SUPPLEMENTUM 422

NET CATION EQUIVALENCY ('BASE BINDING POWER') OF THE PLASMA PROTEINS

A STUDY OF ION-PROTEIN INTERACTION IN HUMAN PLASMA
BY MEANS OF
IN VIVO ULTRAFILTRATION AND EQUILIBRIUM DIALYSIS

by

A. M. VAN LEEUWEN

ACCOMPANIES VOL. 176

ACTA MEDICA SCANDINAVICA

has been published since 1919 as a continuation of Nordiskt Medicinskt Arkiv founded in 1869 by Axel Key The first volume of Acta Medica Scandinavica is therefore numbered LII (52)

The chief editors have been Axel Key 1869—1900, C G Santesson 1901—1915 I Holmgren 1916—1957 and Birger Strandell 1958 to date.

Acta Medica Scandinavica publishes original research papers in the field of internal medicine *Priority will be given to authors from countries which are* represented on the editorial board Contributors from those countries should submit their papers to a representative of the country in question. Contributors from other countries should send their papers to the editor at the address below

Submission of a manuscript for publication will be held to imply that the paper has not been published elsewhere and that, if accepted it will not be republished in any other journal in the same or similar form, without the Editor s written consent

Papers will be published in English, French or German at the authors choice, *followed by a summary in English.*

The manuscripts shall be *in final form as submitted.* They shall be *typewritten* with double spacing and broad left hand margin

If a paper exceeds 16 printed pages, the cost for the excess pages *shall be borne* by the author No paper shall exceed 24 printed pages

Subscription

The annual subscription to the journal *covering two volumes,* each of 6 numbers, *is 140 Sw crowns or U.S $ 27 25 including postage* in the Scandinavian countries and in Holland 120 Sw crowns.

Address for subscriptions and all communications
ACTA MEDICA SCANDINAVICA
P O Box 2052 Stockholm 2

Requests for duplicate copies of numbers that have gone astray can only be considered if made within a fortnight of the receipt of the following number

NET CATION EQUIVALENCY ('BASE BINDING POWER') OF THE PLASMA PROTEINS



From the University Medical Clinic (Head Prof. Dr J. G. G. Borst), Binnengasthuis, Amsterdam

NET CATION EQUIVALENCY
(BASE BINDING POWER)
OF THE PLASMA PROTEINS

A STUDY OF ION-PROTEIN INTERACTION IN HUMAN PLASMA
BY MEANS OF
IN VIVO ULTRAFILTRATION AND EQUILIBRIUM DIALYSIS

by

A. M VAN LEEUWEN

1964
AMSTERDAM

To my parents

1964
SCHELTEMA & HOLKEMA N.V
AMSTERDAM

This publication has been made possible by the financial support of The Netherlands Organisation for the Advancement of Pure Research (Z.W.O.)

Contents

General Introduction

In the course of an earlier investigation in which we assisted GERBRANDY we became increasingly intrigued by the so-called 'base binding power' of the plasma proteins.

In the literature on ion-protein interaction the term 'binding' is not always well defined. It is confusing to read first one author who concludes that Na is bound by plasma proteins, then another who concludes that it is not, and finally find that the authors are in perfect agreement but use the term binding in a different sense.

Apart from its ambiguity as a term, the 'base binding power' of the plasma proteins is characterised by the scarcity of facts concerning its magnitude. The normal value always quoted – 16 mEq/L plasma – is based on a single publication by VAN SLYKE *et al* in 1928. Although in itself an impressive example of detailed and exact experimental evidence, its usefulness must be limited by the fact that the substrates studied were albumin and globulin obtained by ammoniumsulfate fractionation of two horse sera and one batch of pooled human serum. In the light of present knowledge concerning protein chemistry it is questionable whether the results thus obtained are valid for plasma.

This has a bearing on the data concerning the distribution of ions across a semi permeable membrane. The 'base binding power' of the plasma proteins is one of the main factors determining the ion distribution across the capillary wall and the value found by Van Slyke *et al* has been used to calculate the Donnan ratio. But whether the ratio thus obtained is correct is difficult to assess because the inorganic ions have different concentration ratios, and it is unknown which one – if any – of these ratios is identical with the Donnan ratio. The differences in concentration ratio are presumably caused by differences in the nature of the ion-protein bond. Consequently comparison of the concentration ratios observed in dialysis experiments with the Donnan ratio expected provides information concerning the interaction between ion and protein as NORTHROP & KUNITZ could demonstrate for gelatin (1925). A similar approach is possible with the plasma proteins and has indeed been used by GREENE & POWER (1931) but their conclusions are open to criticism because the value of the Donnan ratio for the equilibrium plasma-interstitial fluid was not known then (and is not known now) with sufficient accuracy. This leads back to the questions concerning the nature and the magnitude of the 'base binding power' of the plasma proteins.

If the data are already scarce and inadequate for normal plasma, they are even more so for plasma in disease. But the careful observations of GUTMAN *et al* (1936) appear to justify their negative conclusion concerning the validity of the values given by Van Slyke *et al* in the case of plasma with an abnormal protein composition.

PART III

General Introduction

In the course of an earlier investigation in which we assisted GERBRANDY we became increasingly intrigued by the so-called 'base binding power' of the plasma proteins.

In the literature on ion-protein interaction the term 'binding' is not always well defined. It is confusing to read first one author who concludes that Na is bound by plasma proteins, then another who concludes that it is not, and finally find that the authors are in perfect agreement but use the term binding in a different sense.

Apart from its ambiguity as a term, the 'base binding power' of the plasma proteins is characterised by the scarcity of facts concerning its magnitude. The normal value always quoted – 16 mEq/L plasma – is based on a single publication by VAN SLYKE *et al* in 1928. Although in itself an impressive example of detailed and exact experimental evidence, its usefulness must be limited by the fact that the substrates studied were albumin and globulin obtained by ammoniumsulfate fractionation of two horse sera and one batch of pooled human serum. In the light of present knowledge concerning protein chemistry it is questionable whether the results thus obtained are valid for plasma.

This has a bearing on the data concerning the distribution of ions across a semi permeable membrane. The 'base binding power' of the plasma proteins is one of the main factors determining the ion distribution across the capillary wall and the value found by Van Slyke *et al.* has been used to calculate the Donnan ratio. But whether the ratio thus obtained is correct is difficult to assess because the inorganic ions have different concentration ratios, and it is unknown which one – if any – of these ratios is identical with the Donnan ratio. The differences in concentration ratio are presumably caused by differences in the nature of the ion-protein bond. Consequently comparison of the concentration ratios observed in dialysis experiments with the Donnan ratio expected provides information concerning the interaction between ion and protein as NORTHROP & KUNITZ could demonstrate for gelatin (1925). A similar approach is possible with the plasma proteins and has indeed been used by GREENE & POWER (1931) but their conclusions are open to criticism because the value of the Donnan ratio for the equilibrium plasma-interstitial fluid was not known then (and is not known now) with sufficient accuracy. This leads back to the questions concerning the nature and the magnitude of the 'base binding power' of the plasma proteins.

If the data are already scarce and inadequate for normal plasma, they are even more so for plasma in disease. But the careful observations of GUTMAN *et al* (1936) appear to justify their negative conclusion concerning the validity of the values given by Van Slyke *et al* in the case of plasma with an abnormal protein composition.

The present study is the outcome of an attempt to understand the nature of the base binding power of the plasma proteins and to determine its magnitude both in health and in various states of disease under conditions which imitate the situation *in vivo* as closely as possible This was done by studying the effect of changes in the protein concentration of plasma on the concentrations of Na, K Ca, Mg, Cl and HCO_3 For this purpose we made use of two techniques

In vivo ultrafiltration the principle of which we learned from GERBRANDY The protein concentration of venous plasma is caused to increase by producing venous congestion in the forearm By correlating the change in protein concentration with the concomitant changes in the concentration of the ions mentioned above, information can be obtained concerning the interaction between these ions and the plasma proteins The accuracy required for our purpose made it necessary to study the effect of congestion on the composition of other components of venous blood as well

Equilibrium dialysis in which fresh serum or purified proteins fractions were dialysed *in vitro* at 38 C through a cellulose membrane against synthetic interstitial fluid The difference in ion concentration inside and outside the membrane can be correlated with the protein concentration inside, and the concentration ratio can be calculated for each ion Also the effect of change in pH can be studied

Since the base binding power' of the plasma proteins was obtained by summating the concentrations of the individual cations and substracting the sum concentration of chloride and bicarbonate, the errors of at least 7 individual determinations cumulate Much of our effort had to be devoted to keeping these errors as small as possible this is reflected in the space taken up by the description and discussion of the methods of chemical analysis and of the experimental techniques.

The subject matter will be presented in three parts

Part I is concerned with definitions and with the physico-chemical background of the methods used (chapter I), with data from the literature regarding the base binding power of plasma proteins (for which the term net cation equivalency shall be used), and the concentration ratio of the various small ions across the capillary wall (chapter II).

In Part II we shall describe and discuss methods of chemical analysis (chapter III), the technique of *in vivo* ultrafiltration (chapter IV) and that of equilibrium dialysis (chapter V)

In Part III we will present the results obtained with *in vivo* ultrafiltration (chapter VI) and those obtained with equilibrium dialysis (chapter VII). In chapter VIII the results obtained with both methods are compared and conclusions are drawn with regard to the net cation equivalency of the plasma proteins, the Donnan ratio for the equilibrium plasma – interstitial fluid the protein-bound quantities of the various inorganic ions and the nature of their interaction with protein Chapter IX contains the summing up

All tables with the exception of the last two (chapter IX), have been printed together in a separate booklet, which is added as an appendix.

Some further remarks must be made

Chapter I was written in an attempt to provide the medical reader with sufficient physico-chemical data to understand the limitations of the methods used for studying ion-protein interaction At the same time chapter I should provide the theoretical base

for the experiments and the conclusions to be presented. As a result, the first chapter has become rather extensive, even though far from complete as a physico-chemical introduction. But it will perhaps explain why we find ion-protein binding a fascinating subject.

In this study the attention will be mainly directed to the effect of plasma proteins on the inorganic plasma ions in general, and on the univalent cations and anions in particular. The bivalent cations, notably Ca, have always held most of the attention and the studies concerning the interaction between protein and Ca, recently also Mg, have been reviewed admirably and repeatedly in recent years.

The inorganic ions will be indicated by their atomic symbol. But whereas in the theoretical considerations in chapter I they are provided with the appropriate valency sign, no valency sign will be used in the later chapters where we are concerned with the ions as determined by chemical analysis.

A number of other symbols and abbreviations have been used. These are explained in chapter I and listed on the next page.

On account of our insufficient knowledge of statistical mathematics we have limited our statistical treatment to the calculation of regression lines (method of least squares) and the calculation of standard deviations for a single determination from duplicate determinations (using the equation

$$s^2 = \sum_1 (x_1 - x_2)^2/2n,$$

where n is the number of duplicates). However Dr C. G. G. VAN HERK (Mathematisch Centrum in Amsterdam) was kind enough to analyse statistically the primary information obtained in the *in vivo* ultrafiltration experiments with normal subjects. He has described the results of this analysis separately (see Addendum).

Finally I am most grateful to miss J. J. VAN DAATSELAAR, Mrs. P. C. KAPTEYN-JANSSE and Mrs. C. M. DE SENERPONT DOMIS-THOMASSE for their painstaking chemical analyses. Without their dedication this study would not have been possible. I am also indebted to our biochemist L. A. DE VRIES for always being ready to help and – most important – to listen.

The present study is the outcome of an attempt to understand the nature of the base binding power of the plasma proteins and to determine its magnitude both in health and in various states of disease under conditions which imitate the situation *in vivo* as closely as possible. This was done by studying the effect of changes in the protein concentration of plasma on the concentrations of Na, K, Ca, Mg, Cl and HCO_3. For this purpose we made use of two techniques:

In vivo ultrafiltration, the principle of which we learned from GERBRANDY. The protein concentration of venous plasma is caused to increase by producing venous congestion in the forearm. By correlating the change in protein concentration with the concomitant changes in the concentration of the ions mentioned above, information can be obtained concerning the interaction between these ions and the plasma proteins. The accuracy required for our purpose made it necessary to study the effect of congestion on the composition of other components of venous blood as well.

Equilibrium dialysis in which fresh serum or purified protein fractions were dialysed *in vitro* at 38° C through a cellulose membrane against synthetic interstitial fluid. The difference in ion concentration inside and outside the membrane can be correlated with the protein concentration inside and the concentration ratio can be calculated for each ion. Also the effect of change in pH can be studied.

Since the base binding power of the plasma proteins was obtained by summating the concentrations of the individual cations and substracting the sum concentration of chloride and bicarbonate, the errors of at least 7 individual determinations cumulate. Much of our effort had to be devoted to keeping these errors as small as possible; this is reflected in the space taken up by the description and discussion of the methods of chemical analysis and of the experimental techniques.

The subject matter will be presented in three parts.

Part I is concerned with definitions and with the physico-chemical background of the methods used (chapter I), with data from the literature regarding the base binding power of plasma proteins (for which the term net cation equivalency shall be used), and the concentration ratio of the various small ions across the capillary wall (chapter II).

In Part II we shall describe and discuss methods of chemical analysis (chapter III) the technique of *in vivo* ultrafiltration (chapter IV) and that of equilibrium dialysis (chapter V).

In Part III we will present the results obtained with *in vivo* ultrafiltration (chapter VI) and those obtained with equilibrium dialysis (chapter VII). In chapter VIII the results obtained with both methods are compared and conclusions are drawn with regard to the net cation equivalency of the plasma proteins, the Donnan ratio for the equilibrium plasma – interstitial fluid, the protein bound quantities of the various inorganic ions and the nature of their interaction with protein. Chapter IX contains the summing up.

All tables, with the exception of the last two (chapter IX), have been printed together in a separate booklet which is added as an appendix.

Some further remarks must be made.

Chapter I was written in an attempt to provide the medical reader with sufficient physico-chemical data to understand the limitations of the methods used for studying ion protein interaction. At the same time chapter I should provide the theoretical base

PART 1

List of Abbreviations

Cat, An	= cation, anion
[ ]	= concentration expressed per Kg H_2O
f	= activity coefficient
NCE	= net cation equivalency of proteins (base binding power')
Pr^-	= negative net charge of proteins
RA	= rest anions, *i.e* anions other than chloride, bicarbonate and protein
CatPr AnPr	= cation respectively anion protein-bound in a complex type manner
cat min Cl + HCO_3	= sum concentration of Na, K Ca and Mg minus sum concentration of Cl and HCO_3

The following abbreviations and symbols refer to membrane equilibria

suffix i	= inside *i.e* protein-containing solution
suffix o	= outside *i.e* protein-free solution
r	= Donnan ratio
$R_{cat} = \frac{[Cat]_o}{[Cat]_i}$	= concentration ratio of cation
$R_{an} = \frac{[An]_i}{[An]_o}$	= concentration ratio of anion
ΔCat	= $[Cat]_i - [Cat]_o$

PART 1

CHAPTER I

Physico-chemical introduction to the study of the interaction between inorganic ions and proteins in plasma with a definition of terms

When studying the interaction between proteins and small ions in solutions such as plasma assumptions of a simplifying nature must be made To begin with we will have to consider plasma as being essentially an aqueous solution containing on the one hand the inorganic ions H Na K Ca^{2} Mg^{2} Cl and HCO_3^- and on the other proteins. But other assumptions will have to be made as well and when the nature and limitations of these assumptions are not recognised the interpretation of the experimental data may be seriously in error as the literature reveals. One example is provided by those studies concerning membrane equilibrium in which no distinction is made between the Donnan ratio on the one hand and the concentration ratio of a particular ion on the other Another example is found in the fact that the base binding power of the plasma proteins is quite generally accepted as being identical with the negative net charge of the proteins. This is not so as we hope to show

This chapter is the outcome of an attempt to realise the assumptions underlying the present study and the extent to which they affect the interpretation of the experimental data. We will first consider the characteristics of the plasma constituents with which we are concerned (water ions and proteins), and the interactions between the latter two Ion-protein interaction will then be defined in a manner suited to the purpose of our study and those aspects of membrane equilibria will be discussed which are necessary for the interpretation of our experiments. We will then review the literature concerning on binding by albumin, this being the plasma protein most studied Finally we will consider what information concerning ion-protein binding we may expect to obtain from our experiments with plasma.

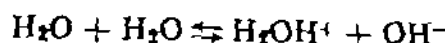
Since a review of general principles is always based on concepts and facts gathered over a long period by a multitude of scientists, we shall refrain from quoting authors in particular unless a definite investigation is at the back of an example or statement. But we should like to mention here BOOIJ (1956) and EDSALL & WYMAN (1958), whose introductions to physical biochemistry have been most helpful.

WATER, IONS AND PROTEINS

WATER

In aqueous solutions the water molecules largely determine the characteristics of the solution. Because of their structure ($O\langle^{H}_{H}$) the molecules act as dipoles and tend to

hang together. Solutes *carrying an electrostatic* charge (ions) attract the water molecules. These arrange themselves around the sites of charge (hydration) and in *doing so diminish* the electrostatic forces acting between the charged particles in solution. H^+ ions (protons) and OH^- *ions* occupy a *special* position owing to the fact that water molecules are not completely stable. The negative pole *of a* H_2O *molecule (the side where the O-atom is* situated) tends to attract H^+ ions from the positive poles of neighbouring H_2O molecules (the side where the H atoms are located). In reality therefore water must be described *by the equilibrium equation*



$$H_2O + H_2O \rightleftarrows H_2OH^+ + OH^-$$

In pure water the amount of (H_2OH^+) and (OH^-) is extremely small. If H^+ ions are added they will attach themselves *to* H_2O *molecules and move through the solution* from one molecule to another. This explains the high mobility of H^+ ions and their important role in aqueous solutions.

Concerning concentrations

Water being the solvent, the amount of solutes must be expressed per litre or per Kg H_2O, preferably the latter because then the solvent can be considered in terms of molecules (1000 g H_2O = 55.55 moles H_2O). *Concentrations per Kg* H_2O *are indicated by placing* the molecular symbol of the solute between brackets [ ]. When molecular interactions are studied the concentration of the solutes is expressed in (milli)moles per Kg H_2O; *when ion-ion interactions are investigated* the concentration is expressed *in (milli)* equivalents per Kg H_2O.

The concentration of the H^+ ions is not expressed as such but in pH values. For *practical* purposes one can consider pH = *—log* $[H^+]$.

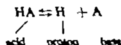
IONS

An ion *is a substance which carries an electrostatic charge.* Classical examples are the inorganic ions. These are atoms which have acquired a positive or negative charge by losing or taking up electrons. Positively charged ions tend to form stable crystals with *negatively charged ions (for example* NaCl, $KHCO_3$, $CaCl_2$). *But when dissolved* in water these substances dissociate in their component ions because of the hydration and the resulting 'insulation' of the ions. The relative independence thus obtained by the individual ions (Na^+, K^+, Ca^{2+}, Cl^-) *is manifest from the fact that when an electrostatic* field is applied to the solution the component ions of the original substance move in opposite direction and may leave the solution at the electrodes. Hence the name 'electrolytes' and the denomination of positively charged ions by the *term cation and of negatively charged* ions by the term *anion*. Considered individually ions have a remarkable freedom of movement, but as a group they are subject to the restriction that the total positive (cationic) charge must equal the total negative (anionic) *charge in the solution* *('law of electroneutrality')*.

The inorganic ions may differ considerably in their behaviour towards other substances as a result of differences in sign and magnitude *(valency) of their electrostatic* charge *and in the size of the ion*. An example is the tendency to attract water molecules, which is strongest when the ion radius is small and the valency high.

Ionogenic group – Acid – Base

The term ion or ionic substance has been extended to groups of atoms which have acquired a positive or negative electrostatic charge by a process of association or dissociation. An arrangement of atoms which is capable of doing so is indicated by the term ionogenic group. The essential point about ionogenic groups is that they may or may not carry an electrostatic charge, according to the circumstances. Examples are provided by the acids and bases. BRØNSTED defines an acid as a substance which becomes a base by losing an H^+-ion (protondonator), a base as a substance which becomes an acid by taking up an H^+-ion. The H^+-ion has a central position in the definition and the relation between acid and base can best be described by the equation



$$HA \rightleftharpoons H^+ + A^-$$

In this example the acid is the ionogenic substance. The ease with which an acid will dissociate into H^+ and A^- is indicated by the value of the *dissociation constant* which is defined by the equation

$$\frac{[H^+][A^-]}{[HA]} = K_a$$

The higher the value of K_a the "stronger" the acid HA (and the "weaker" the base A^-). Solutions containing approximately equal amounts of a weak acid and its base (or a weak base and its acid) tend to keep the H^+-ion concentration constant (buffer solutions). To fit the pH notation the equilibrium is in the case of buffers generally described as

$$pH = pK_a + \log \frac{[A^-]}{[HA]} \quad (\text{where } pK_a = -\log K_a)$$

The dissociation constant is not really a constant. It depends on the temperature of the solution, on the characteristics of the other solutes, on the "density" of acid molecules (their molal concentration). When a molecule contains more than one ionogenic group the tendency to dissociate is strongly influenced by the spatial arrangement of the groups.

Spatial arrangement of ionogenic groups

Its influence may be illustrated by comparing phosphoric acid and citric acid. Both carry 3 acid groups and can therefore donate 3 H^+-ions. But in the case of phosphoric acid all the ionogenic —OH groups are attached to the same P atom, whereas in the case of citric acid each of the ionogenic —COOH groups is connected to a different C atom, which are themselves separated by another C atom. The dissociation of the first group and the concomitant acquirement of a negative charge will in the case of phosphoric acid strongly depress the tendency to dissociation for the remaining groups. But in the case of citric acid the distance is sufficiently large to diminish the influence of one group on the others. The point is illustrated by comparing for both substances the constants of each of the three steps of dissociation.

	K_1	K_2	K_3
Phosphoric acid (at 18°)	1.1×10^{-2}	7.5×10^{-8}	4.8×10^{-13}
Citric acid (at 25°)	8.4×10^{-4}	1.8×10^{-5}	4×10^{-6}

(Taken from *Handbook of Chemistry and Physics* 30th ed.)

This example stresses the fact that in the case of large molecules carrying several univalent dissociated ionogenic groups the molecule should be regarded as being essentially a number of univalent ions instead of one single polyvalent ion

Ionic substances which can simultaneously carry a positive and a negative charge (amphoteric substances) – Net charge

The classic example is provided by the amino acids Amino acids are essentially a combination of a —COOH group a —NH_2 group and an alkyl group which may or may not carry other ionogenic groups The —NH_2 group is in the α position *i.e.* it is connected to the same C atom as the —COOH group and whereas the carboxyl group is a rather strong acid the —αNH_2 group is a weak base If we consider the simplest amino acid, glycin we find that at very low pH values the carboxyl group is not dissociated and therefore carries no charge, but that the amino group is associated with a H^+ ion and thus carries a positive charge (—H_2NH^+) (fig. 1) When we add NaOH

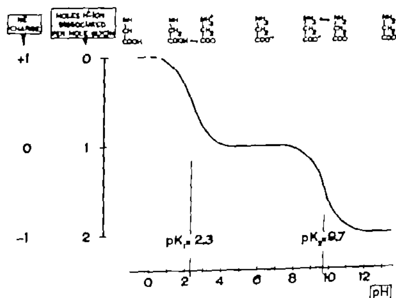


Fig. 1 Schematic representation of titration curve of glycin.

till the pH is increased to a value higher than the pK of the —COOH group but still below the pK of the —H_2NH^+ group the amino acid ion still carries a positive charge, but in addition now also a negative one If finally the pH increases to values well above the value of the pK of the —H_2NH^+ group the latter loses its H^+-ion and the amino acid ion carries only a negative charge Thus while first one and then the second H^+ ion is dissociated the charge on the ion changes from one unit of positive charge to a combination of one unit of positive and one unit of negative charge, and finally to one unit of negative charge The arithmetic sum of positive and negative charges on the ion – its *net charge* – therefore changes from $+1 \rightarrow$ zero $\rightarrow -1$ Fig. 1 is a (schematic) repre-

sentation of such a titration curve. At the ordinate are indicated both the number of H -ions dissociated per molecule and the net charge of the molecule. The pH trajectory over which the net charge remains zero is the *iso-electric pH* trajectory. In this – simple – case it is a pH range, but if the number and variety of ionogenic groups on a molecule increase the pH range over which the amphoretic ion has a net charge of zero narrows down until for proteins it has become one fairly circumscript pH value, the iso-electric pH (IEP). At this point the effect of an amphoteric ion on the balance between the total cationic and anionic charges in the solution is the same as that of a neutral molecule.

A number of amino acids carries other ionogenic groups beside the primary —COOH and the —αNH_2 group. Because of the difference in dissociation constants of these groups the titration curves of polypeptides take on a rather complicated form and the number of H -ions which can successively be dissociated with increasing pH can become very large.

The size of ionic substances – Filtrable and non-filtrable ions – Free filtrable ion

Ionic size acquires special importance when an ionic solution is surrounded by a porous membrane. If the pores are sufficiently narrow the small ions can pass through the membrane but the macromolecular ions can not. We will therefore designate ions as *filtrable* or *non-filt able* according to whether their size does or does not permit them to pass through the pores of such a semipermeable membrane (collodion, capillary membrane).

Although filtrable, ions such as Na Ca^{++} etc. are refrained from passing through semipermeable membranes when they have become attached to non-filtrable substances. The term 'filtrable' refers therefore to the potential rather than to the actual behaviour of an ion. filtrable ion not bound by non-filtrable substance will be indicated as 'free'

PROTEINS

Proteins are highly intricate, amphoteric macromolecules. They are ionic substances, but size and structure put them in a class apart.

Because of their size the proteins are perfect examples of non-filtrable ions. They can therefore be separated from the filtrable ions present in the same solution by means of dialysis or filtration through a suitable semipermeable membrane. However the presence of protein on only one side of the membrane causes the filtrable ions to distribute themselves in an unequal fashion between the two solutions d vided by the membrane. The resulting Donnan equilibrium is of considerable interest and will be discussed in the third part of this chapter

The structure of the proteins allows for an enormous diversity in number nature and spatial arrangement of ionogenic groups. The backbone of the protein molecules is a chain of peptide bonds which are formed by the reaction of the primary —COOH group of one amino acid w th the —αNH_2 group of another. From this backbone structure the remaining parts of the amino acid molecules project as side chains. Since the bond between the nitrogen atom and the carbon atom carrying the side chain can rotate in regard to the plane in which the other atoms of the peptide bond are situated, the chain of peptide bonds can assume different shapes. The two main variations are the linear arrangement found in proteins l'ke keratin and fibrin, and the spiral or helix arrangement

which is typical for the globular proteins to which all other plasma proteins belong Especially with the latter type of proteins intricate three-dimensional structures of great molecular weight can be formed by alternating clockwise and counterclockwise rotating helices *with parts that show a linear* arrangement

Among the factors responsible for the shape of a protein molecule are the nature and position of the side chains Those carrying ionogenic groups tend to project into the surrounding water *whereas the purely aliphatic side chains strive to get away from the* water and huddle together in the centre of the protein molecule, probably creating a hydrophobic core in some of the proteins.

The ionogenic groups most frequently *encountered* are *the carboxyl groups and the* amino groups, but imidazole, phenolic, guanidin and sulfhydryl groups are present as well The values of the dissociation constants of these groups range from about 10^{-3} to 10^{-12} which makes the titration curves of most proteins rather complicated Of special interest is the spacing of the ionogenic groups on the protein surface When the carboxyl groups for instance are wide apart they represent at a pH value of 7 4 truly univalent sites of charge. But when there is a high density of carboxyl groups in a certain area of the protein surface this area will at pH 7 4 show a considerable accumulation of negative electrostatic charge and the effect on the surrounding solution will be comparable to that of bi or even trivalent sites of charge Although in the case of the plasma proteins practically nothing is known about spatial arrangements of ionogenic groups these different possibilities must be kept in mind when considering ion protein interaction

Quite a different force, but one which contributes greatly to the shape of the protein molecules, results from the strong dipole nature of the peptide bond, where the >NH group acts as positive and the >C=O group as negative pole. This favours the attraction of the ~NH group in one peptide bond by the >C=O group in another peptide bond (located for instance in a parallel coil of the helix structure) by making them share the H-atom of the amino group. These hydrogen bonds are very unstable and easily influenced by factors like temperature, concentration of inorganic ions in the solution, the pH etc.

All this explains why most of the plasma proteins are very sensitive to relatively small alterations in the composition and temperature of the solution Furthermore both the number of and variety in plasma proteins are remarkably large. They have molecular weights ranging from about 40 000 to about 1 000 000 their isoelectric points range from 2.7 to 7 3 they have a carbohydrate content varying from insignificant to something like 40 % and a lipid content varying from *1 to 99 % of the molecular weight* coeruloplasmin has a highly specific affinity for Cu^{2+} transferrin for Fe^{3} The amount of knowledge which has been gathered concerning the plasma proteins is impressive Yet the number of plasma proteins which are now well defined as to composition, structure and physico-chemical characteristics is still small notwithstanding highly ingenious research in which the most varied chemical and physical methods are *used This is due* to the difficulty of obtaining a pure protein without altering its properties to such an extent that it becomes a mere caricature of itself The fact that albumin is the one plasma protein about which *most is known is partly* due to its robustness But the example of albumin may also serve to show that *in vitro* experiments with purified proteins are not necessarily representative for the behaviour of the protein as present in plasma. FOSTER *(1960) in discussing* the purity of albumin preparations remarks upon the fact that even in the best crystallised preparations the albumin was found to contain one or a few

moles of fatty acid contamination per mole of protein and that the complete removal of these by special techniques results in a protein preparation which crystallizes with difficulty. Apparently the contamination with fatty acids modifies the physical behaviour of the plasma albumin and Foster concludes by suggesting that certain differences observed in behaviour between bovine and human albumin may reside in differences in the level of fatty acids contamination. Contamination is the operative word here. It suggests a nuisance – which, of course, it is to physical chemists who are in the first place interested in defining the characteristics of pure substances. But the biologist might with equal right consider the removal of the last molecules of 'contaminating' fatty acids the final step in the process of divesting the plasma albumin from its biological characteristics.

Clearly caution is needed when using results obtained in experiments with purified proteins to explain phenomena observed in the study of plasma. With this restriction the results obtained with albumin preparations are of considerable interest and will be reviewed in the fourth part of this chapter.

DISCUSSION OF THE INTERACTION BETWEEN SMALL IONS AND PROTEINS AND DEFINITION OF THE TERMS USED TO DESCRIBE THIS INTERACTION

INTERACTION BETWEEN SMALL IONS (SALT TYPE OF INTERACTION)

Dissociation

In water the component ions of an electrolyte tend to dissociate *i.e.* to be separated by interposition of water molecules. For strong electrolytes (such as NaCl) this dissociation is considered to be complete, *i.e.* all ions are separated by water molecules. However the ions still influence each other through electrostatic forces and as a result a solution of strong electrolytes behaves differently from what the theory of complete dissociation leads one to expect.

Activity – Activity coefficient – Ionic strength

All theoretical considerations concerning the properties of a solution are based on the behaviour of an ideal gas. The molecules of such a hypothetical gas are *inert to each other* and consequently have complete freedom of movement as long as they do not collide. An ideal gas obeys strictly the dictates of the general gas equation. By assuming the solutes in a solution to behave as the *molecules* of an ideal gas, characteristics of the solution such as osmotic force can be considered in terms of the gas equation. However the more concentrated the *solutions studied*, the more the experimental values for freezing point etc. tend to deviate from the *values calculated* by inserting the molar concentrations of the solute in the equations derived from gas kinetics. Since freezing point, vapour pressure, conductivity (*in the case of electrolytes*) depend on the number of 'free' solute particles per unit volume the observed deviation suggests that there are less 'free' particles than one would expect from the molar concentration and the theory of complete dissociation of salts. This phenomenon can be explained as follows. No solute is completely inert, least of all the ions in an electrolyte solution. The insulating effect of the water molecules diminishes the electrostatic attraction to the extent that

the ions no longer adhere spatially but some electrostatic attraction between the ions will remain until their concentration becomes infinitely low Conversely the higher their concentration the more the ions will interact the solution will behave less and less like an ideal gas because for each ion the freedom of movement is increasingly restricted. The effect is the same as when there would be a decrease in the number of completely independent particles per unit volume

One can therefore reconcile the theory of molecular kinetics with the experimental data by considering the hypothetical quantity of completely independent solute particles per unit volume instead of the molar concentrations obtained by chemical analysis. This hypothetical quantity which makes the experimental data fit in with the theory of ideal gas behaviour has been termed by LEWIS *the activity* of a solute Its relation to the concentration is given by the *activity coefficient f* where f = activity/concentration By definition for all solutions, f = 1 0 at infinite dilution From the above considerations it must be concluded that *for an electrolyte solution the activity coefficient is related to – and thus a measure for – the interaction between the ions* The stronger the interaction the lower the activity coefficient of the solution The magnitude of the latter depends therefore on the concentration of the ions, on their valencies, on the temperature of the solution to name the most important factors

The large effect of valency is demonstrated by the following data from LEWIS and RANDALL (1923). At 25°C and a molal concentration of 0 1 the value of f is for NaCl 0.798, for $BaCl_2$ 0.501 for $MgSO_4$ 0 166(!).

A measure for the concentrations and valencies of all ions present in a solution is found in the *ionic strength* of that solution another useful concept of Lewis.

The ionic strength (μ) is defined by the equation

$$\mu = \tfrac{1}{2}\Sigma C_a Z_a^2 + C_b Z_b^2 + \quad C_n Z_n^2$$

where C indicates *molar* concentrations, Z valency and the suffix a, b, n the various types of ion present in the solution. For plasma the ionic strength is generally calculated at 0.16 on the assumption that in regard to ionic strength the proteins act as univalent ions the number of which is equal to the numerical value of protein net charge

Determination of activity coefficient

For solutions containing one or two types of electrolytes the value of f has been determined experimentally from measurements of freezing point, vapour pressure etc. It is also possible to estimate f with the aid of equations derived by DEBYE and HÜCKEL. In these equations figure such factors as ionic strength, ion valency ion diameter dielectric constant of solvent etc For dilute solutions (> 0 1 M/L) containing not too many different ions f can be estimated from ionic strength and valencies by means of a relatively simple equation At higher concentrations and for polyvalent ions the Debye Hückel equations become very complicated and the values calculated less accurate.

Until now we have discussed the activity coefficient of a solution For a single type of ion f can be estimated in two ways. The first one rests on the assumption proposed by MACINNES that the activities of K^+ and of Cl^- ions are equal and have the same value as the activity found for a solution of KCl One can then calculate the activity coefficient for other types of ion by a process of comparison when it is further assumed *that in a dilute solution containing various electrolytes the activity coefficient of any ion depends*

solely on the ionic strength of the solution The latter assumption was made by LEWIS, who stressed its hypothetical nature and made the restriction that a solution can no longer be considered dilute when its ionic strength increases beyond values of a few hundredths to a few tenths, according to the valency of the ions It should be noted that Lewis studied solutions containing no macromolecules.

The second way of measuring the activity coefficient of a single type of ion is provided by the principle of the ion-specific electrodes. Essentially these contain the parent atoms of the particular ion for which they are sensitive. When placed in a solution containing this ion an equilibrium will be established between the rates at which ions turn into atoms and vice versa. A change in the activity of the ions in the solution will therefore cause a corresponding change in the rate at which electrons are released from (or taken up by) the electrode. This phenomenon can be measured by incorporating solution and ion-specific electrode in a measuring circuit which contains a potentiometer a reference electrode in a reference solution, and a liquid junction between the two solutions. If the potentiometer is to indicate correctly the changes in potential at the measuring electrode, then the potentials at the reference electrode and at the junction must have a constant value regardless of the solutions studied. This condition is not easily fulfilled. There are more difficulties.

> The need to define scale with the aid of standard solutions indicates that no absolute alues for ion activity can be obtained from electrometric measurements. Even the interpretation of changes in potential in terms of changes in ion activity is fraught with theoretical difficulties. Accordingly the number of assumptions necessary is already large in the case of simple salt solutions. When electrolyte solutions containing protein are studied, the uncertainties become still larger if only because apparently nothing definite is know about the effects which the presence of proteins as such has on the junction potential and on the measuring electrode. Furthermore when glass-electrodes are used (such as the modern H -ion- and Na -ion electrodes) it must be realised that these are not really specific Apart from the systematic errors, the accidental errors are such that in measurements of this type the standard deviation of duplicate determination is seldom smaller than 2 %.

For these reasons it must be doubted whether it is at present possible to estimate in mixed electrolyte solutions containing protein the activity of any ion with an accuracy better than 3 %. This estimate of error is probably an optimistic one BATES (1954) concludes that electrometic pH measurements cannot truthfully be interpreted in terms of H -ion activity

Assumptions concerning ion activities in interstitial fluid and plasma

A study of the literature reveals that for practical purposes the inorganic ions in interstitial fluid are generally considered to be completely dissociated, and to have an activity coefficient of about 0.7 to 0.8. Furthermore although its ionic strength is about 0 16, interstitial fluid is considered sufficiently dilute a solution that ionic strength and valencies fully determine the activity of the arious types of ion.

Finally when plasma and interstitial fluid are compared (as in the study of membrane equilibria) it is customary to consider the contribution of proteins to ionic strength to be negligible This assumption is based on the concept that the effect of protein net charge on ionic strength is that of an equivalent amount of univalent ions. As a consequence of these assumptions the activity coefficient of the arious types of filtrable ion

the ions no longer adhere spatially but some electrostatic attraction between the ions will remain until their concentration becomes infinitely low. Conversely the higher their concentration the more the ions will interact: the solution will behave less and less like an ideal gas because for each ion the freedom of movement is increasingly restricted. The effect is the same as when there would be a decrease in the number of completely independent particles per unit volume.

One can therefore reconcile the theory of molecular kinetics with the experimental data by considering the hypothetical quantity of completely independent solute particles per unit volume instead of the molar concentrations obtained by chemical analysis. This hypothetical quantity which makes the experimental data fit in with the theory of ideal gas behaviour has been termed by LEWIS *the activity* of a solute. Its relation to the concentration is given by the *activity coefficient f* where f = activity/concentration. By definition for all solutions $f = 1.0$ at infinite dilution. From the above considerations it must be concluded that *for an electrolyte solution the activity coefficient is related to – and thus a measure for – the interaction between the ions*. The stronger the interaction the lower the activity coefficient of the solution. The magnitude of the latter depends therefore on the concentration of the ions, on their valencies, on the temperature of the solution, to name the most important factors.

The large effect of valency is demonstrated by the following data from LEWIS and RANDALL (1923). At 25°C and a molal concentration of 0.1 the value of f is for NaCl 0.798, for $BaCl_2$ 0.501, for $MgSO_4$ 0.166(I).

A measure for the concentrations and valencies of all ions present in a solution is found in the *ionic strength* of that solution, another useful concept of Lewis.

The ionic strength (μ) is defined by the equation

$$\mu = \tfrac{1}{2}\Sigma C_a Z_a^2 + C_b Z_b^2 + \ldots C_n Z_n^2$$

where C indicates *molar* concentrations, Z valency and the suffix a, b, ... n the various types of ion present in the solution. For plasma the ionic strength is generally calculated at 0.16 on the assumption that in regard to ionic strength the proteins act as univalent ions the number of which is equal to the numerical value of protein net charge.

Determination of activity coefficient

For solutions containing one or two types of electrolytes the value of f has been determined experimentally from measurements of freezing point, vapour pressure etc. It is also possible to estimate f with the aid of equations derived by DEBYE and HÜCKEL. In these equations figure such factors as ionic strength, ion valency, ion diameter, dielectric constant of solvent etc. For dilute solutions (> 0.1 M/L) containing not too many different ions f can be estimated from ionic strength and valencies by means of a relatively simple equation. At higher concentrations and for polyvalent ions the Debye-Hückel equations become very complicated and the values calculated less accurate.

Until now we have discussed the activity coefficient of a solution. For a single type of ion f can be estimated in two ways. The first one rests on the assumption proposed by MACINNES that the activities of K^+ and of Cl^--ions are equal and have the same value as the activity found for a solution of KCl. One can then calculate the activity coefficient for other types of ion by a process of comparison when it is further assumed *that in a dilute solution containing various electrolytes the activity coefficient of any ion depends*

solely on the ionic strength of the solution. The latter assumption was made by Lewis, who stressed its hypothetical nature and made the restriction that a solution can no longer be considered dilute when its ionic strength increases beyond values of a few hundredths to a few tenths, according to the valency of the ions. It should be noted that Lewis studied solutions containing no macromolecules.

The second way of measuring the activity coefficient of a single type of ion is provided by the principle of the ion-specific electrodes. Essentially these contain the parent atoms of the particular ion for which they are sensitive. When placed in a solution containing this ion an equilibrium will be established between the rates at which ions turn into atoms and vice versa. A change in the activity of the ions in the solution will therefore cause a corresponding change in the rate at which electrons are released from (or taken up by) the electrode. This phenomenon can be measured by incorporating solution and ion-specific electrode in a measuring circuit which contains a potentiometer a reference electrode in a reference solution, and a liquid junction between the two solutions. If the potentiometer is to indicate correctly the changes in potential at the measuring electrode, then the potentials at the reference electrode and at the junction must have a constant value regardless of the solutions studied. This condition is not easily fulfilled. There are more difficulties.

The need to define scale with the aid of standard solutions indicates that no absolute values for ion activity can be obtained from electrometric measurements. Even the interpretation of changes in potential in terms of changes in ion activity is fraught with theoretical difficulties. Accordingly the number of assumption necessary is already large in the case of simple salt solutions. When electrolyte solutions containing protein are studied, the uncertainties become still larger if only because pparently nothing definite is known about the effects which the presence of proteins in such has on the junction potential and on the measuring electrode. Furthermore when glass-electrodes are used (such as the modern H -ion- and Na -ion electrodes) it *must* be realised that these are not really specific. Apart from the systematic errors, the accidental errors are such that in measurements of this type the standard deviation of duplicate determination is seldom smaller than 2

For these reasons it must be doubted whether it is at present poss ble to estimate in mixed electrolyte solutions containing protein the activity of any ion with an accuracy better than 3 . This estimate of error is probably an optimistic one Bates (1954) concludes that electrometic pH measurements cannot truthfully be interpreted in terms of H -ion activity

Assumptions concerning ion activities in interstitial fluid and plasma

A study of the literature reveals that for practical purposes the inorganic ions in interstitial fluid are generally considered to be completely dissociated, and to have an activity coefficient of about 0.7 to 0.8 Furthermore although its ionic strength is about 0.16 interstitial fluid is considered sufficiently dilute a solution that ionic strength and valencies fully determine the activity of the various types of ion.

Finally when plasma and interstitial fluid are compared (as in the study of membrane equilibria) it is customary to consider the contribution of proteins to ionic strength to be negligible. This assumption is based on the concept that the effect of protein net charge on ionic strength is that of an equivalent amount of univalent ions. As a consequence of these assumptions the activity coefficient of the various types of filtrable ion

the ions no longer adhere spatially but some electrostatic attraction between the ions will remain until their concentration becomes infinitely low. *Conversely the higher their concentration the more the ions will interact: the solution will behave less and less like an ideal gas because for each ion the freedom of movement is increasingly restricted.* The effect is the same as when there would be a decrease in the number of completely independent particles per unit volume.

One can therefore reconcile the theory of molecular kinetics with the experimental data by considering the *hypothetical quantity of completely independent solute particles* per unit volume instead of the molar concentrations obtained by chemical analysis. This hypothetical quantity *which makes the experimental data fit in with the theory of* ideal gas behaviour has been termed by LEWIS *the activity* of a solute. Its relation to the concentration *is given by the activity coefficient f*, where f = activity/concentration. By definition for all solutions $f = 1.0$ at infinite dilution. From the above considerations *it must be concluded that for an electrolyte solution the activity coefficient is related to – and thus a measure for – the interaction between the ions.* The stronger the interaction *the lower the activity coefficient of the solution.* The magnitude of the latter depends therefore on the concentration of the ions, on their valencies, on the temperature of the *solution, to name the most important factors.*

The large effect of valency is demonstrated by the following data from LEWIS and RANDALL (1923). At 25°C and a molal concentration of 0.1 the value of f is for NaCl 0.798, for BaCl 0.501, for $MgSO_4$ 0.166(!).

A measure for the concentrations and valencies of all ions present in a solution is found *in the ionic strength of that solution, another useful concept of Lewis.*

The ionic strength (μ) is defined by the equation

$$\mu = \tfrac{1}{2}\Sigma C_a Z_a^2 + C_b Z_b^2 + \dots C_n Z_n$$

where C indicates *molar* concentrations, Z valency and the suffix a, b, ... n the various types of ion present in the solution. For plasma the ionic strength is generally calculated at 0.16 on the assumption that in regard to ionic strength the proteins act as univalent ions the number of which is equal to the numerical value of protein net charge.

Determination of activity coefficient

For solutions containing one or two types of electrolytes the value of *f has been determined experimentally* from measurements of freezing point, *vapour pressure* etc. It is also possible to estimate f with the aid of equations derived by DEBYE and HÜCKEL. In these equations figure such factors as *ionic strength, ion valency, ion diameter*, dielectric constant of solvent etc. For dilute solutions (> 0.1 M/L) containing not too many different ions *f can be estimated from ionic strength* and valencies by means of a relatively simple equation. At higher concentrations and for polyvalent ions the Debye-Hückel equations become *very complicated* and the values calculated less accurate.

Until now we have discussed the activity coefficient of a solution. For a single type of ion *f can be estimated in two ways.* The first one rests on the assumption proposed by MACINNES that the activities of K^+ and of Cl^- ions are equal and have the same value *as the activity found for a solution of KCl.* One can then calculate the activity coefficient for other types of ion by a process of comparison when it is further assumed *that in a dilute solution containing various electrolytes the activity coefficient of any ion depends*

increasing dissociation. The dissociation constant was discussed before. But when cations other than H -ions are studied interest is mostly in the tendency to associate. This tendency is indicated by the reciprocal of the dissociation constant, which is called association constant or binding constant. The latter term results from the fact that physical chemists generally use the term binding without further qualification to indicate complex-binding.

Determination of complex-bound ion

When in an electrolyte solution part of a particular type of ion is present in a soluble complex-compound, the concentration obtained by chemical analysis is the sum of the dissociated and the complex-bound (non-dissociated) fraction. Since ionic strength – and hence ion activity – is related to the dissociated ions only the activity of the particular type of ion will be less than its concentration would lead one to expect. In other words, the presence of complex-bound ion manifests itself in a decrease of the activity coefficient of the particular ion below the value calculated with the aid of Debye-Hückel equations on the assumption that all ions are dissociated. In theory therefore the amount of ion that is complex-bound by a certain substance can be determined by comparing the activity coefficients in two solutions which are identical otherwise but one of which contains the particular substance. Among the earliest and most impressive examples of the use of this method for studying ion-protein interaction are the experiments of NORTHROP & KUNITZ (1926, 1928) on ion 'binding' by gelatin, and those of HENRIQUES (1929) on the interaction between carbon dioxide derivatives and haemoglobin. In the case of plasma it is rather difficult to measure accurately the activity of a particular type of ion (see previous section). But in the third part of this chapter we will see that the ion distribution observed in experiments in which plasma is subjected to equilibrium dialysis against (synthetic) interstitial fluid can give information concerning complex binding in plasma. That is, provided a number of assumptions is made concerning the ion-activities in plasma (see page 11).

THE EFFECT WHICH A HIGH DENSITY OF ELECTROSTATIC CHARGE ON THE SURFACE OF THE PROTEIN MOLECULES WILL HAVE ON THE IONS IN THE SURROUNDING SOLUTION

We have assumed that the electrostatic interaction between the ions of a dissociated salt is non-specific, and that their activity can be predicted from the valencies of the ions and the ionic strength of the solution, provided the latter is sufficiently dilute. The assumptions are based on the concept of a homogenous distribution of electrostatic charge throughout the solution. This concept is wrong when the solution contains protein molecules with a high density of electrostatic charges in certain areas of their surface. In that case ions carrying an opposite charge accumulate around the areas of high charge, and the higher the valency of the counter ions the stronger they will be attracted. In this connection it must be realised that local accumulations of dissociated groups may exist while the *net* charge of the protein is zero. If the density of the surface charge becomes sufficiently high these counter ions will be much more restricted in their freedom of movement – *i.e.* in their activity – than the ionic strength, calculated for the solution as a whole, would lead one to expect. This extra decrease in activity

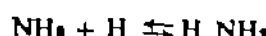
are considered to be equal in plasma and in interstitial fluid, as long as the proteins interact with the filtrable ions in solution in the manner of the ions of a fully dissociated salt. At the ionic strength of plasma this electrostatic interaction is considered to be non specific.

INTERACTION BETWEEN NEUTRAL MOLECULES AND IONS

Complex-binding

In contrast to the long-range effects of electrostatic forces, the forces acting between a neutral molecule and an ion are very strong when the two are at a distance of a few Å, but their strength decreases with extreme rapidity when the distance increases. In this type of interaction we need therefore distinguish only two possible states. Either the ion is associated with and attached to the molecule or it is not. In the latter case the interaction between molecule and ion may be neglected in comparison to the electrostatic interactions between the ions. The ammonia molecule is an example of a neutral molecule which can associate with an ion.

The nitrogen atom shares 3 of the 5 electrons present in its outer electron shell each with one H-atom. The two remaining electrons ("lone pair") tend to associate with cations which are one or two electrons short, such as an H^+ ion



$$NH_3 + H^+ \rightleftarrows H^+NH_3$$

Ammonia is therefore a proton acceptor (a base), and since the ammonia molecule is neutral the result of association is a positively charged ammonium ion. The same applies to the amino group.

This type of ion formation is an example of association by coordination or *complex binding*. Molecules containing a central atom with one or more lone pairs of electrons (nitrogen, oxygen, sulphur) tend to form such coordination compounds.

By its nature complex binding allows for selective attraction of ions, and this selectivity can become very pronounced indeed when several such groups are suitably arranged spatially. Such arrangements partly explain the high affinity of certain molecular structures for ions such as Zn^{2+}, Cu^{2+}, Mn^{2+} and Fe^{2+}. That spatial arrangement can induce a certain selectivity even in electrostatic attraction is demonstrated by the affinity of citrate ions for Ca^{2+} ions.

In contrast to the univalent cations the bivalent Ca^{2+}-ion "fits" between two of the three dissociated carboxyl groups of citrate, and is so firmly incorporated in the citrate molecule that both the two carboxyl groups and the Ca^{2+} ion cease to contribute to the ionic strength of the solution (HASTINGS *et al.* 1934).

Associations which result in the formation of similar ringlike structures are indicated by the term *chelation*.

Association or binding constant

In the case of complex binding the interaction between molecule and ion practically ceases to exist when the two dissociate. Complex binding can therefore be described in terms of the Mass Law and an equilibrium constant calculated. When the interest is in the tendency to dissociate (as is the case with the acid complex-compounds —COOH and $—H^+NH_2$) the equation is written so that the value of the constant increases with

increasing dissociation. The dissociation constant was discussed before. But when cations other than H -ions are studied interest is mostly in the tendency to associate. This tendency is indicated by the reciprocal of the dissociation constant, which is called association constant or binding constant. The latter term results from the fact that physical chemists generally use the term binding without further qualification to indicate complex-binding.

Determination of complex-bound ion

When in an electrolyte solution part of a particular type of ion is present in a soluble complex-compound, the concentration obtained by chemical analysis is the sum of the dissociated and the complex-bound (non-dissociated) fraction Since ionic strength – and hence ion activity – is related to the dissociated ions only the activity of the particular type of ion will be less than its concentration would lead one to expect. In other words, the presence of complex-bound ion manifests itself in a decrease of the activity coefficient of the particular ion below the value calculated with the aid of Debye-Hückel equations on the assumption that all ions are dissociated. In theory therefore the amount of ion that is complex bound by a certain substance can be determined by comparing the activity coefficients in two solutions which are identical otherwise but one of which contains the particular substance. Among the earliest and most impressive examples of the use of this method for studying ion-protein interaction are the experiments of NORTHROP & KUNITZ (1926 1928) on ion binding by gelatin, and those of HENRIQUES (1929) on the interaction between carbon dioxide derivatives and haemoglobin. In the case of plasma t is rather difficult to measure accurately the activity of a particular type of ion (see previous section). But in the third part of this chapter we will see that the ion distribution observed in experiments in which plasma is subjected to equilibrium dialysis against (synthetic) interstitial fluid can give information concerning complex-binding in plasma. That is, provided a number of assumptions is made concerning the ion-activities in plasma (see page 11).

THE EFFECT WHICH A HIGH DENSITY OF ELECTROSTATIC CHARGE ON THE SURFACE OF THE PROTEIN MOLECULES WILL HAVE ON THE IONS IN THE SURROUNDING SOLUTION

We have assumed that the electrostatic interaction between the ions of a dissociated salt is non-specific, and that their activity can be predicted from the valencies of the ions and the ionic strength of the solution, provided the latter is sufficiently dilute. The assumptions are based on the concept of a homogenous distribution of electrostatic charge throughout the solution. This concept is wrong when the solution contains protein molecules with a high density of electrostatic charges in certain areas of their surface. *In that case ions carrying* an opposite charge accumulate around the areas of high charge, and the higher the valency of the counter ions the stronger they will be attracted. In this connection it must be realised that local accumulations of dissociated groups may exist while the *net* charge of the protein is zero If the density of the surface charge becomes sufficiently high these counter ions will be much more restricted in their freedom of movement – *i e* in their activity – than the ionic strength calculated for the solution as a whole, would lead one to expect. This extra decrease in activity

is then not the result of complex binding and cannot *therefore be described by the* stoechiometric relations of the Mass Law But, especially in solutions such as plasma, it is difficult to distinguish between true complex binding and the *type of strong electro*static attraction mentioned above In the latter case also a certain selectivity exists in that ion characteristics such as valency diameter of hydrated ion etc. modify the strength of the attraction

This adsorption of small ions by strong electrostatic forces on a surface is an old concept. It explains quite a number of phenomena in colloid chemistry (see for instance the work of Bungenberg de Jong and co-workers on the association colloids – BUNGENBERG DE JONG *et al* in *Colloid Science* vol II 1949) The concept got somewhat out of fashion in the American literature on protein chemistry but in recent years the experience with the synthetic ion exchangers – which have a very high density of electrostatic charge – has been a reminder of the importance of adsorption phenomena in the vicinity of accumulated electrostatic charge.

It is *customary* in *calculations of the ionic strength of plasma either to neglect the charge* on the proteins or to consider its effect to be no more than that of a number of univalent ions As a consequence an extra decrease in ion-activity due to the unsuspected presence of areas with a *high* density of electrostatic charge will be interpreted as indicating true complex binding

INTERACTION BETWEEN PROTEIN AND WATER MOLECULES

Points of charge on protein molecules attract water molecules in the same manner as do *small cations and anions and there are indications that up to two molecules of water are* bound per point of charge (WAUGH 1954) Although it is the general custom to calculate all concentrations per unit weight of water (obtaining the latter by drying a sample of protein solution to constant weight) it might be asked whether this value should not be corrected for the water bound to cationic and anionic substances in much the same manner as the ion-concentration is corrected to obtain ion activity (see for instance GROLLMAN 1931) But the difficulties involved are considerable one being how to distinguish between an increase in the activity of solute and a decrease in activity of water For the present to express the concentration of solute per unit weight of water remains the best quantitative description of the components of a solution.

INTERACTION BETWEEN THE VARIOUS PROTEIN MOLECULES

Proteins with different *IEP* might be *expected to interact with each other* if the *pH* value of the solution is in the intermediate range But for the plasma proteins we have found *no data concerning this effect In experiments* with plasma it is generally neglected

DEFINITION OF THE TERM BINDING
DISTINCTION IN 'SALT TYPE' AND COMPLEX TYPE'

From the previous sections it will be clear that in plasma *the interactions between* proteins and small ions must range *from the typical* salt-type of interaction in which the ion is fully dissociated, to the *typical complex-binding in which* the ion is no longer

dissociated. Between these extremes a variety of interactions must exist which are of an intermediate type in that they result in a more or less pronounced restriction of ion movement. For practical purposes it is necessary to reduce the number of possible interactions to a few main types. The most convenient simplification is to distinguish two types according to whether or not the activity of the ion decreases below the value which would be calculated from the ionic stength of the solution and the valencies of the ions with the aid of the Debye Hückel equations.

In this study the term *binding* will be used to indicate any attraction between small, *i.e.* filtrable ions and proteins regardless of the nature and the strength of the attracting forces. Two types will be distinguished.

(*a*) The binding will be considered to be of the *salt-type* when in the presence of the protein the activity coefficient of the filtrable ion under consideration is not different from that in a solution of equal ionic composition and ionic strength but containing no protein. In this type of binding the ion is considered to be dissociated from the protein. The (electrostatic) attraction between protein and ion is in this case essentially non-specific and only demands that the charge on the protein is – statistically – balanced by an equivalent charge of opposite sign in the surrounding solution.

(*b*) The binding will be considered to be of the *complex-type* when it leads to a decrease in the activity coefficient of the filtrable ion below the value expected in an electrolyte solution which is identical but for the presence of protein. This decrease in activity coefficient can be the result of association of the ion because of true complex-binding or chelation, or it may be the expression of an inordinate restriction of ion-movement as a result of strong electrostatic attraction. In a complicated solution such as plasma these modes of interaction cannot at present be distinguished from each other.

The above resolution of all possible interactions in two types is at the back of most discussions on ion-protein binding. Apparently, however, the pragmatic nature of the distinction is seldom realised. As a consequence decreases of ion activity below the value expected from ionic strength are interpreted as indicative of true complex-binding. Whereas this may be sufficiently near the truth for those cations which show an excessive tendency to complex-formation such as Cu^{2+}, Zn^{2+}, Fe^{3+}, it must be doubted whether it is correct in the case of Ca^{2+} and Mg^{2+} and in the case of the univalent cations. If strong electrostatic charges on the protein molecules severely depress the activity of the counter-ions but the latter remain dissociated, calculations based on the assumption that binding is either of the salt-type or of the complex-type, will automatically result in estimating a (hypothetical) fraction of the ions as truly complex-bound and the remainder as fully dissociated in the manner of the ions of a salt. The interpretation of the values thus obtained in terms of a definite number of "binding sites" and their use for calculating association constants must be viewed with suspicion in the case of Ca^{2+} and Mg^{2+} and certainly in the case of Na^{+} and K^{+}.

All this does not detract from the practical value of the schematic distinction of two types of binding. It is very useful when analysing the differences in behaviour of the various ions towards the proteins. But in view of the numerous assumptions made, values calculated for ion bound in respectively a salt-type or a complex type manner have a relative rather than an absolute significance.

This restriction must be kept in mind when reading the following pages.

is then not the result of complex binding and cannot therefore be described by the stoechiometric relations of the Mass Law. But, especially in solutions such as plasma, it is difficult to distinguish between true complex binding and the type of strong electrostatic attraction mentioned above. In the latter case also a certain selectivity exists in that ion characteristics such as valency, diameter of hydrated ion etc. modify the strength of the attraction.

This adsorption of small ions by strong electrostatic forces on a surface is an old concept. It explains quite a number of phenomena in colloid chemistry (see for instance the work of Bungenberg de Jong and co-workers on the association colloids – BUNGENBERG DE JONG *et al.* in *Colloid Science* vol. II 1949). The concept got somewhat out of fashion in the American literature on protein chemistry, but in recent years the experience with the synthetic ion exchangers – which have a very high density of electrostatic charge – has been a reminder of the importance of adsorption phenomena in the vicinity of accumulated electrostatic charge.

It is customary in calculations of the ionic strength of plasma either to neglect the charge on the proteins or to consider its effect to be no more than that of a number of univalent ions. As a consequence an extra decrease in ion activity due to the unsuspected presence of areas with a high density of electrostatic charge will be interpreted as indicating true complex binding.

INTERACTION BETWEEN PROTEIN AND WATER MOLECULES

Points of charge on protein molecules attract water molecules in the same manner as do small cations and anions and there are indications that up to two molecules of water are bound per point of charge (WAUGH 1954). Although it is the general custom to calculate all concentrations per unit weight of water (obtaining the latter by drying a sample of protein solution to constant weight), it might be asked whether this value should not be corrected for the water bound to cationic and anionic substances in much the same manner as the ion-concentration is corrected to obtain ion activity (see for instance GROLLMAN 1931). But the difficulties involved are considerable, one being how to distinguish between an increase in the activity of solute and a decrease in activity of water. For the present, to express the concentration of solute per unit weight of water remains the best quantitative description of the components of a solution.

INTERACTION BETWEEN THE VARIOUS PROTEIN MOLECULES

Proteins with different IEP might be expected to interact with each other if the pH value of the solution is in the intermediate range. But for the plasma proteins we have found no data concerning this effect. In experiments with plasma it is generally neglected.

DEFINITION OF THE TERM BINDING
DISTINCTION IN SALT TYPE AND COMPLEX TYPE

From the previous sections it will be clear that in plasma the interactions between proteins and small ions must range from the typical salt-type of interaction, in which the ion is fully dissociated, to the typical complex-binding in which the ion is no longer

sociated filtrable anion. According to whether respectively more cation or more anion is bound in a salt-type manner the protein net charge is respectively negative or positive.

It must be stressed that the above definition of net charge differs from the one given in the first part of this chapter where net charge was defined as the arithmetic sum of the positive and the negative charges on an amphoteric substance. The latter definition is the more correct one theoretically But since in a solution such as plasma the net charge of the protein can only be measured indirectly from its effect on the surrounding dissociated filtrable cations and anions, the definition accepted for the purpose of our study has practical advantage. Since the electrostatic attraction responsible for the salt-type of binding cannot be described in terms of the Mass Law the value calculated for protein net charge in the indirect manner described above does not necessarily represent an identical number of charged ionogenic groups on the protein molecule.

Relation between NCE and negative net charge in plasma
In the physiological pH range more cation than anion is bound by plasma protein In the case of plasma we will therefore have to use the terms net cation equivalency' and 'negative net charge The relation between the two can be described as follows (NCE) = (negative net charge) + (complex bound cation) (complex bound anion), all expressed in equivalent concentrations.

THE EFFECT OF PROTEIN ON THE DISTRIBUTION OF FILTRABLE IONS ACROSS A SEMIPERMEABLE MEMBRANE AND THE USE OF THIS EFFECT IN THE STUDY OF PROTEIN BOUND ION

MEMBRANE EQUILIBRIA

When two identical electrolyte solutions, containing filtrable ions only are separated by a semipermeable membrane the osmotic pressure of the solution and the activity of each ion will be equal on both sides of the membrane. The ratio of the activities is therefore 1.000. If then a non-filtrable ionic substance, such as protein, is added to one of the two solutions the osmotic and electrostatic equilibrium is disturbed. After a while equilibrium will be re-established by water molecules moving through the semi permeable membrane into the protein solution, and by filtrable ions moving in the opposite direction. In the new state of equilibrium the increase in hydrostatic pressure of the protein containing solution balances the combined osmotic effect of the presence of protein and of the redistribution of filtrable ions. At the same time the unilateral presence of non-filtrable electrostatic charge is compensated for by an unequal activity of the filtrable ions in the two solutions. The activity rat os therefore differ from 1.000 Since for thermodynamical reasons the product of the activities of filtrable cation and filtrable anion must, at equilibrium, be the same in both solutions, while simultaneously electroneutral ty should exist in each solution, the change in activity ratio is in opposite directions for cations and for anions. S gn and magnitude of the non-filtrable electrostatic charge (net charge in case of amphoteric substances) determine in what direction, and how much, the activity ratios will differ from 1.000. The unequal distribution of the filtrable ions will also manifest itself in a potential difference between the two solutions (membrane potential)

DONNAN has been the first to demonstrate these characteristics of membrane equilibria

DEFINITION OF THE TERMS PROTEIN NET ION EQUIVALENCY AND PROTEIN NET CHARGE AS THEY ARE USED IN THIS STUDY – INTRODUCTION OF THE TERM NET CATION EQUIVALENCY' (NCE) INSTEAD OF THE TERM BASE BINDING POWER

The resultant or 'net effect of proteins on the cation-anion diagram of an electrolyte solution

In an electrolyte solution protein contributes to both the cationic and anionic properties of the solution The net effect of this contribution can be measured by subtracting from one another the experimentally determined sum concentrations of the filtrable cations and the filtrable anions The value thus obtained must be equal to the difference between the total amount of protein bound cation and the total amount of protein-bound anion regardless of the nature of the binding. When both cations and anions are bound in a salt type manner only then the difference between their respective sum concentrations relates to fully dissociated ions and it will therefore be equal to protein net charge But when cation and/or anion is bound in a complex type manner as well, then the difference between the sum concentrations of filtrable cation and of filtrable anion includes non-dissociated ion and is therefore not equal to protein net charge

For plasma, where the resultant effect of protein is that of an anion the difference between the concentration of filtrable cations and that of filtrable anions as found by chemical analysis has been termed base binding power by Van Slyke and co-workers. Later in order to avoid the ambiguous term base MANERY (1954) introduced the term cation equivalency

Because no clear distinction was made between salt and complex type of ion-protein binding base binding power' is generally considered as being identical with net charge. Furthermore, the terms base binding power and cation equivalency fail to stress the net character of the quantity to which they refer In this study therefore the following terms will be used

Net ion equivalency of the proteins This is the difference between protein-bound cation and protein-bound anion regardless of the nature of the binding. It is measured by subtracting from one another the concentrations of the filtrable cations and the filtrable anions as obtained by chemical analysis According to whether the proteins bind more cation or more anion we will use the terms net cation equivalency or net anion equivalency Since in plasma the proteins bind cations in excess of anions we will use the term *net cation equivalency which will be abbreviated to NCE.* This term is synonymous with the term base binding power The terms *cation (anion) equivalency* will indicate the amount of filtrable cation (anion) bound by protein regardless of the nature of the bond By definition

(NCE) = (cation equivalency) minus (anion equivalency) all expressed in equivalent concentration

Net charge of the proteins This is quantitatively equal to the difference between the equivalent amounts of cation and of anion that are protein-bound in a salt-type of manner By definition therefore protein net charge has a value equivalent to the difference between the concentration of fully dissociated filtrable cation and that of fully dis

sociated filtrable anion. According to whether respectively more cation or more anion is bound in a salt type manner the protein net charge is respectively negative or positive.

It must be stressed that the above definition of net charge differs from the one given in the first part of this chapter where net charge was defined as the arithmetic sum of the positive and the negative charges on an amphoteric substance. The latter definition is the more correct one theoretically. But since in solution such as plasma the net charge of the proteins can only be measured indirectly from its effect on the surrounding dissociated filtrable cations and anions, the definition accepted for the purpose of our study has practical advantage. Since the electrostatic attraction responsible for the salt-type of binding cannot be described in terms of the Mass Law the value calculated for protein net charge in the indirect manner described abov does not necessarily represent an identical number of charged ionogenic groups on the protein molecules.

Relation between NCE and negative net charge in plasma

In the physiological pH range more cation than anion is bound by plasma protein. In the case of plasma we will therefore have to use the terms net cation equivalency' and negative net charge. The relation between the two can be described as follows (NCE) = (negative net charge) + (complex-bound cation) — (complex bound anion), all expressed in equivalent concentrations.

THE EFFECT OF PROTEIN ON THE DISTRIBUTION OF FILTRABLE IONS ACROSS A SEMIPERMEABLE MEMBRANE AND THE USE OF THIS EFFECT IN THE STUDY OF PROTEIN-BOUND ION

MEMBRANE EQUILIBRIA

When two identical electrolyte solutions, containing filtrable ions only are separated by a semipermeable membrane the osmotic pressure of the solution and the activity of each ion will be equal on both sides of the membrane. The ratio of the activities is therefore 1.000. If then a non-filtrable ionic substance, such as protein, is added to one of the two solutions the osmotic and electrostatic equilibrium is disturbed. After a while equilibrium will be re-established by water molecules moving through the semipermeable membrane into the protein solution, and by filtrable ions moving in the opposite direction. In the new state of equilibrium the increase in hydrostatic pressure of the protein containing solution balances the combined osmotic effect of the presence of protein and of the redistribution of filtrable ions. At the same time the unilateral presence of non-filtrable electrostatic charge is compensated for by an unequal activity of the filtrable ions in the two solutions. The activity ratios therefore differ from 1.000. Since for thermodynamical reasons the product of the activities of filtrable cation and filtrable anion must, at equilibrium, be the *same in both* solutions, while simultaneously electroneutrality should exist in each solution, the change in activity ratio is in opposite directions for cations and for anions. Sign and magnitude of the non-filtrable electrostatic charge (net charge in case of amphoteric substances) determine in what direction and how much, the activity ratios will *differ from* 1.000 The unequal distribution of the filtrable ions will also manifest itself in a potential difference between the two solutions (membrane potential).

DONNAN has been the first to demonstrate these characteristics of membrane equilibria

DEFINITION OF THE TERMS PROTEIN 'NET ION EQUIVALENCY' AND PROTEIN NET CHARGE AS THEY ARE USED IN THIS STUDY – *INTRODUCTION OF THE* TERM 'NET CATION EQUIVALENCY (NCE) INSTEAD OF THE TERM BASE BINDING POWER

The resultant or 'net effect of proteins on the cation-anion diagram of an electrolyte solution

In an electrolyte solution protein contributes to both the cationic and anionic properties of the solution The net effect of this contribution can be measured by subtracting from one another the experimentally determined sum concentrations of the filtrable cations and the filtrable anions The value thus obtained *must be equal to the difference* between the total amount of protein bound cation and the total amount of protein bound anion regardless of the nature of the binding. When both cations and anions are bound in a salt type manner only then the difference between their respective sum concentrations relates to fully dissociated ions and it will therefore be equal to protein net charge. But when cation and/or anion is bound in a complex type manner as well, then the difference between the sum concentrations of filtrable cation and of filtrable anion includes non-dissociated ion and is therefore not equal to protein net charge

For plasma, where the resultant effect of protein is that of an anion the difference between the concentration of filtrable cations and that of filtrable anions as found by chemical analysis has been termed base binding power by Van Slyke and co-workers. Later in order to avoid the ambiguous term base MANERY (1954) introduced the term cation equivalency

Because no clear distinction was made between salt and complex type of ion-protein binding base binding power is generally considered as being identical with net charge. Furthermore the terms base binding power and cation equivalency fail to stress the net character of the quantity to which they refer In this study therefore the following terms will be used

Net ion equivalency of the proteins This is the difference between protein bound cation and protein bound anion regardless of the nature of the binding. It is measured by subtracting from one another the concentrations of the filtrable cations and the filtrable anions as obtained by chemical analysis According to whether the proteins bind more cation or more anion we will use the terms net cation equivalency or net anion equivalency Since in plasma the proteins bind cations in excess of anions we will use the term *net cation equivalency which will be abbreviated to NCE* This term is synonymous with the term base binding power The terms *cation (anion) equivalency* will indicate the amount of filtrable cation (anion) bound by protein regardless of the nature of the bond By definition

(NCE) = (cation equivalency) minus (anion equivalency) all expressed in equivalent concentration

Net charge of the proteins This is quantitatively equal to the difference between the equivalent amounts of cation and of anion that are protein-bound in a salt type of manner By definition therefore protein net charge *has a value equivalent to the difference* between the concentration of fully dissociated filtrable cation and that of fully dis

out that values calculated with the aid of equations which are based on the assumptions mentioned above can only have a relative significance.

The determination of the Donnan ratio for the equilibrium plasma-synthetic interstitial fluid as observed in equilibrium dialysis experiments

The concentration ratios for the univalent cations and anions observed in experiments of this type (see chapter II) suggest that for normal plasma the value of the Donnan ratio must be somewhere between 0.93 and 0.98. If the Donnan ratio is to be of any use in determining whether and if so to what extent, the univalent cations and anions in plasma are complex-bound, then the value of the Donnan ratio has to be known within 0.005 as we will see in the discussion of our own experiments.

Measurement of the membrane potential (from which the Donnan ratio can be calculated by means of one of the equations derived by Donnan) is not at present sufficiently accurate because of systematic and accidental errors.

The following example may serve to show the accuracy needed. If the Donnan ratio were 0.950 the membrane potential should be 1.31 mV (at 38°C). For Donnan ratio of 0.960 the relevant equation indicates a potential of 1.05 mV. One will be hard put to detect such small potential difference with certainty. The possibility of large systematic errors is indicated by the recent study of SALMINEN (1961). He measured among other things the potential difference between normal human serum and its ultrafiltrate. The average value was found to be about 9 mV, which would correspond with a Donnan ratio of about 0.69. The value is absurd in view of the fact that in the same experiments the concentration ratio of Na was on the average 0.92.

The relative inaccuracy is still greater when the Donnan ratio is calculated from the ion activities as measured in plasma respectively in interstitial fluid by means of ion-specific electrodes. In this case the errors of two measurements are summated. The errors to be expected have been indicated already (page 11).

Some authors, notably GREENE & POWER (1931), assumed that at physiological pH values none of the plasma Cl was bound in a complex-type manner. The concentration ratio of Cl was therefore accepted as being equal to the Donnan ratio. But the results of recent experiments with purified albumin (to be discussed in the next part of this chapter) suggest that part of the plasma Cl is after all complex-bound.

VAN SLYKE *et al.* calculated r from data which at first sight are obtained with relative ease. In their masterly treatise on the factors controlling the electrolyte and water distribution in the blood (1923) they derived a number of equations among which one defining the relation between Donnan ratio, protein net charge and concentration ratio of ion for the equilibrium serum-interstitial fluid. But the authors neglected the fact that a certain amount of the ions present in plasma is complex-bound by protein and the equations must therefore be reconsidered.

An additional difficulty is the fact that only the "base binding power" as determined by Van Slyke *et al.* in 1928 is available for calculating protein net charge. Apart from the fact that it remains to be seen whether protein net charge can be obtained from "base binding power", it will be shown (chapter II) that the value found by Van Slyke *et al.* can no longer be accepted unless substantiated by fresh experimental evidence.

In view of all this it is not surprising that even for the *in vitro* equilibrium between plasma (or serum) and synthetic interstitial fluid the precise value of the Donnan ratio remains a matter of conjecture (MANERY 1954).

(DONNAN & HARRIS 1911) With the aid of thermodynamic considerations published by GIBBS DONNAN could derive equations which describe the osmotic and electrostatic phenomena existing across a semipermeable membrane (DONNAN 1911 1924) For the derivation of the fundamental equations the reader is referred to Donnan s publications. *Donnan expressly stated that these equations* are *only* valid for equilibrium conditions, and that – since they are based on thermodynamical considerations – the equations are concerned with activities not with concentrations

LOEB (1922) has been the first to apply the Donnan theory to biological problems. Since then many have followed who made use of it in the study of osmotic or electrostatic phenomena. Since in this study we are concerned with *the latter we will neglect* the osmotic effect of membrane equilibrium. This is permissible because we will only *consider equilibrium conditions, i.e conditions in which* osmotic and electrostatic equilibrium exists simultaneously The two phenomena can then be considered separately

THE DONNAN RATIO AND ITS USEFULNESS FOR ANALYSING ION-PROTEIN INTERACTION

The Donnan ratio

When plasma is in equilibrium with interstitial fluid through a semipermeable membrane, we can consider plasma as interstitial fluid to which protein has been added. According to the theory of Donnan the ratio of the ion activities will for this equilibrium be defined by the following equation

$$r = \frac{f[H^+]_o}{f[H^+]_i} = \frac{f[Na^+]_o}{f[Na^+]_i} = \frac{f[K^+]}{f[K^+]_i} = \frac{\sqrt{f[Ca^{2+}]_o}}{\sqrt{f[Ca^{2+}]_i}} = \quad = \frac{f[Cl^-]_i}{f[Cl^-]_o} = \frac{f[HCO_3^-]_i}{f[HCO_3^-]_o}$$

where the suffix i indicates the protein containing solution ('inside'), the suffix o the non-protein solution ('outside)

*The symbol r is called the Donnan ratio Its magnitude depends for any given equi*librium on the protein concentration and the total concentration of filtrable electrolytes (see later) For the present the most important aspect of the Donnan ratio is that with its aid the activity ratio can be calculated for each of the filtrable ions. *Conversely we* need only measure the activity ratio for one of the ions to know the Donnan ratio

The relation between Donnan ratio and concentration ratios

In the case of the equilibrium between plasma and interstitial fluid the Donnan theory can be made to apply to concentration ratios if it is assumed that plasma and interstitial fluid have the same *ionic strength, and therefore the same activity coefficients for the* dissociated ions (see page 11) The concentration ratio of an ion will then equal its activity ratio and hence the Donnan ratio *provided that the plasma proteins only interact with the particular ion in a salt type manner* When the proteins bind part of the ion in a complex type manner the concentration ratio of the ion will differ from the Donnan ratio and consequently from the concentration ratios of the ions which are bound in a salt-type manner only

The above mentioned simplification has been generally made in previous studies concerning the ion distribution between plasma and interstitial fluid. Since no *workable alternative presents itself* we will make the same simplification But it should be pointed

he amount of a particular type of filtrable cation or anion associated with protein in a salt-type manner are necessarily subject to the same restriction. Since at physiological pH the net charge of the serum proteins has a negative sign, the result will be that only cations will appear to be protein-bound in a salt-type manner and calculations concerning the extent of this type of binding will only reveal the amount of cation bound in excess of anion. This restriction does not apply to the calculation of complex-bound cation or anion, since these are not subject to the law of electroneutrality

Fig. 2 is also amiss in that no filtrable complex-bound substances are shown, although plasma contains small amount of such compounds (Ca-citrate and possibly some Ca-phosphate). Since these are not subject to the law of electroneutrality their concentration is the same on both sides of the membrane and before we can apply the equations which are to follow the total concentration of the cation or anion involved should be corrected for the amount present in such filtrable complex-compounds.
However since in our dialysis experiments to be reported later the total concentration of these substances must have been less than 0.1 m mole per litre we have followed previous authors in neglecting their presence.

Calculation of Donnan ratio

In the outward compartment depicted in fig. 2 both cations and anions are present as freely filtrable ion only (Cat^+_o, An^-_o). In the protein-containing compartment anion is also present as freely filtrable anion only (An^-_i), but cation is present both as filtrable ion (Cat^+_i) and complex-bound by protein (Cat Pr). The greatest part of Cat^+_i is balanced by An^-_i, the remainder by the negative charges on the protein molecules (Pr^-). All quantities are expressed in equivalent concentration and it must be realised that Pr^- is equivalent to the amount of univalent cation balancing the anionic groups on the protein molecules.

Since the Donnan ratio is only affected by the activity of the ions in solution, we find

$$r = \frac{f[Cat^+]_o}{f[Cat^+]_i} = \frac{f[An^-]_i}{f[An^-]_o}$$

And since we have assumed ionic strength to be equal in both solutions

$$r = \frac{[Cat^+]_o}{[Cat^+]_i} = \frac{[An^-]_i}{[An^-]_o} \qquad (1)$$

In the situation depicted in fig. 2 the analytically determined ratio of the cation, *i.e. the concentration ratio (hence forward to be indicated by the symbol* R_{cat}), will be smaller than r. This is evident from the following equation

$$R_{cat} = \frac{[Cat]_o}{[Cat]_i} = \frac{[Cat^+]_o}{[Cat^+]_i + [Cat\ Pr]} < r \qquad (2)$$

Acknowledging the fact that $[Cat^+]_o = [Cat]_o$ because the ions are considered to be fully dissociated in the absence of protein, we find by substitution from (1) and (2)

$$r = \frac{[Cat]_o \times R_{cat}}{[Cat]_o - [Cat\ Pr] \times R_{cat}} \qquad (3)$$

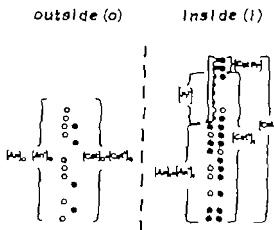
As in our opinion the approach made by Van Slyke and associates appears at present to be the most promising one, we will reconsider their equations for calculating the value of r while making allowance for the presence of complex bound ion. Once the Donnan ratio is known, the extent to which an ion is complex bound can be calculated from its concentration ratio by means of relatively simple equations. This was demonstrated already by Northrop and Kunitz.

For what follows we are greatly indebted to VAN SLYKE *et al.* (1923), NORTHROP & KUNITZ (1925, 1926) and GREENE & POWER (1931) whose publications have contributed largely to our understanding.

EQUATIONS FOR CALCULATING THE DONNAN RATIO AND THE QUANTITY AND QUALITY OF PROTEIN BOUND ION

Schematic representation of equilibrium between serum and interstitial fluid

The scheme to be used in the following discussion (fig. 2) is a simplified representation of serum in equilibrium with interstitial fluid across a semipermeable membrane. It is simplified in that only one type of filtrable cation and anion is represented and in that



LEGEND: Cat. — amount determined by chemical analysis; Cat. A — amount present as ions; Cat.Pr — amount complex bound by protein; Pr⁻ — sum of anionic groups on protein

Fig. 2. Schematic representation of equilibrium existing between serum and interstitial fluid across a semipermeable membrane.

protein is represented as carrying a negative charge only. The first simplification is not a serious one, because no matter how many different cations and anions are present, individually they have to conform to the same laws. The second simplification will not affect the calculation of the Donnan ratio, as the latter only depends on the *net* charge of the proteins. But at the same time this implies that a macromolecule carrying an identical amount of negative and of positive charges will not influence the partition of ions across a semipermeable membrane. In this respect it cannot be distinguished from a macromolecule which carries no charge at all. It follows that, in as far as negatively charged ionogenic groups on the protein molecules are compensated by positively charged groups on the same molecules, they cannot be detected by studying the distribution of filtrable ions across a semipermeable membrane. All conclusions concerning

ed that owing to the assumption of equal ionic strengths for plasma and for interstitial fluid no activity coefficients appear in the equation. I the second part of this chapter it has been argued that the cations balancing the negatively charged sites on the protein surface may have lower activity coefficient than the other ions in the solution. In that case different activity coefficients would have to be inserted in equation (6) for $[An^-]_i$ and for $[Pr^-]$ in order to obtain the correct Donnan ratio. Neglect of the activity coefficients will lead to an erroneously low value for Conversely when is known and equation (6) is used to calculate the negative net charge, $[Pr^-]$, the latter will be calculated too low. Although therefore in equation (6) the error due to the possible presence of AnPr is minimized, we have introduced with Pr^- new difficulty

We have now three equations from which in theory r can be obtained equation (3) or (5) can be used should it be known which fraction of cation or anion is complex-bound by protein equation (6) is available in case the concentration of filtrable anion in the protein-containing solution is known as well as protein net charge.

Calculation of quality and quantity of protein-bound ion

Since R_{cat} respectively R_{an} are related to r and to Cat Pr respectively An Pr (see equations (3) and (5)), the complex bound fraction of an ion can be calculated when both its concentration ratio and the Donnan ratio are known. The equations differ somewhat according to whether the ion is univalent or bivalent.

We will first consider the univalent cation in fig. 2. By chemical analysis we find $[Cat]_o$ and $[Cat]_i$. *The difference between these two values to be indicated as Δ Cat is related to the total amount of cation bound by protein.* But whereas the latter is equal to $[Cat\ Pr] + [Pr^-]$, it will be seen that $\Delta\ Cat = [Cat]_i - [Cat]_o = [Cat\ Pr] + [Cat^+]_i -$ $- [Cat^+]_o$, and Δ Cat therefore underestimates the total amount of protein-bound cation. From fig. 2 it will be evident that the underestimate is equal to $[An^-]_o - [An^-]_i$, which according to equation (1) can also be written as $(1 - r)\ [An^-]_o$. And since $[An^-]_o$ must be equal to $[Cat^+]_o$ we find that

$$\text{protein-bound univalent cation} = \Delta\ Cat + (1 - r)\ [Cat]_o \qquad (7)$$

Provided r is known we should therefore be able to calculate the total amount of cation which is protein-bound. However as we discussed already the amount thus calculated is a minimum value since Pr^- stands in fact for the negative *net* charge. The total negative charge of the proteins may be considerably larger but the difference escapes detection, since it is compensated by the positive charge of the proteins which we ha e no way of measuring. The amount of anion which is protein-bound in a salt-type manner will therefore also remain unknown.

No such difficulties are encountered in the following equations which relate r and R to *the amount f ion which is complex-bound by protein.*

By rearranging equation (3) we find for univalent cation

$$[Cat\ Pr] = \frac{r - R_{cat}}{r \times R_{cat}}\ [Cat]_o = \frac{r - R_{cat}}{r}\ [Cat]_i \qquad (8)$$

And by rearranging equation (5) for univalent anion

$$[An\ Pr] = (R_{an} - r)\ [An]_o = \frac{R_{an} - r}{r}\ [An]_i \qquad (9)$$

If we now consider the anions we find that according to our scheme the concentration ratio of the anions should give us the Donnan ratio – as Greene and Power assumed But our scheme differs from reality since at physiological pH some Cl (about 1—3 %) is probably complex bound by serum albumin

In the inside compartment the anion concentration found by chemical analysis should then represent $[An^-]_i + [An\,Pr]$ Consequently the *concentration ratio* R_{an} must be larger than the Donnan ratio since

$$R_{an} = \frac{[An]_i}{[An]_o} = \frac{[An^-]_i + [An\,Pr]}{[An^-]_o} > r \tag{4}$$

By following the same reasoning as in the case of the cations we can derive the following equation

$$r = \frac{[An]_o \times R_a \; - [An\,Pr]}{[An]_o} \tag{5}$$

Equations (3) and (5) can only give us r if for a given ion the concentration in the non protein solution the concentration ratio and the amount that is complex bound in the *protein solution are known*

If this is not the case then another approach is necessary At the normal pH of serum [An Pr] will certainly be smaller than [Cat Pr], and – provided [An Pr] is not more than 4 % of $[An^-]_i$ and $[Pr^-]$ can be determined accurately – it should be possible to calculate r with acceptable accuracy (*i e* to within 0 002) in the following manner

$$r = \frac{[Cat^+]_o}{[Cat^+]_i} = \frac{[An^-]_i}{[An^-]_o}$$

but also

$$\frac{[Cat^+]_o}{[Cat^+]_i} = \frac{[An^-]_o}{[An^-]_i + [Pr^-]} \quad \text{(see fig. 2)}$$

therefore

$$r^2 = \frac{[An^-]_o}{[An^-]_i + [Pr^-]} \times \frac{[An^-]_i}{[An^-]_o}$$

and thus

$$r = \sqrt{\frac{[An^-]_i}{[An^-]_i + [Pr^-]}} \tag{6}$$

As An^-_i now figures in both numerator and denominator the effect of a systematic error due to inserting $[An]_i$ instead of $[An^-]_i$ will be minimised. When $[An]_i$ is 4 % larger than $[An^-]_i$ because of the presence of complex-bound anion the *insertion of* $[An]_i$ in equation (6) will at normal plasma conditions, cause r to be calculated about 0 002 *too high.*

Equation (6) is similar to the corresponding equation given by Van Slyke *et al* (1923) and Hastings *et al* (1927) It differs in that it stresses the fact that it is concerned with *dissociated* ions only

Equation (6) must be used with caution

When more than one type of anion is present in the solution the sum of the equivalent concentrations of all dissociated anions must be used ($\Sigma[An^-]$). The error resulting from the practical necessity to use $[An]_i$ *instead of* $[An^-]_i$ has already been mentioned Finally it should be remember

ed that owing to the assumption of equal ionic strengths for plasma and for interstitial fluid no activity coefficients appear in the equation. In the second part of this chapter it has been argued that the cations balancing the negatively charged sites on the protein surface may have a lower activity coefficient than the other ions in the solution. In that case different activity coefficients would have to be inserted in equation (6) for $[An^-]_i$ and for $[Pr^-]$ in order to obtain the correct Donnan ratio. Neglect of the activity coefficients will lead to an erroneously low value for r. Conversely when r is known and equation (6) is used to calculate the negative net charge, $[Pr^-]$, the latter will be calculated too low. Although therefore in equation (6) the error due to the possible presence of AnPr is minimized, we have introduced with Pr^- a new difficulty.

We have now three equations from which in theory r can be obtained: equation (3) or (5) can be used should it be known which fraction of cation or anion is complex bound by protein; equation (6) is available in case the concentration of filtrable anion in the protein-containing solution is known as well as protein net charge.

Calculation of quality and quantity of protein-bound ion

Since R_{cat} respectively R_{an} are related to r and to Cat Pr respectively An Pr (see equations (3) and (5)), the complex-bound fraction of an ion can be calculated when both its concentration ratio and the Donnan ratio are known. The equations differ somewhat according to whether the ion is univalent or bivalent.

We will first consider the univalent cation in fig. 2. By chemical analysis we find $[Cat]_o$ and $[Cat]_i$. *The difference between these two values, to be indicated as Δ Cat, is related to the total amount of cation bound by protein.* But whereas the latter is equal to $[Cat\ Pr] + [Pr^-]$, it will be seen that $\Delta\ Cat = [Cat]_i - [Cat]_o = [Cat\ Pr] + [Cat^+]_i - [Cat^+]_o$, and Δ Cat therefore underestimates the total amount of protein-bound cation. From fig. 2 it will be evident that the underestimate is equal to $[An^-]_o - [An^-]_i$, which according to equation (1) can also be written as $(1 - r)\,[An^-]_o$. And since $[An^-]_o$ must be equal to $[Cat^+]_o$ we find that

$$\text{protein-bound univalent cation} = \Delta\ Cat + (1 - r)\,[Cat]_o \qquad (7)$$

Provided r is known we should therefore be able to calculate the total amount of cation which is protein-bound. However, as we discussed already, the amount thus calculated is a minimum value since Pr^- stands in fact for the negative *net* charge. The total negative charge of the proteins may be considerably larger, but the difference escapes detection since it is compensated by the positive charge of the proteins which we have no way of measuring. The amount of anion which is protein-bound in a salt-type manner will therefore also remain unknown.

No such difficulties are encountered in the following equations which relate r and R to *the amount of ion which is complex-bound by protein.*

By rearranging equation (3) we find for univalent cation

$$[Cat\ Pr] = \frac{r - R_{cat}}{r \times R_{cat}}\,[Cat]_o = \frac{r - R_{cat}}{r}\,[Cat]_i \qquad (8)$$

And by rearranging equation (5) for univalent anion

$$[An\ Pr] = (R_{an} - r)\,[An]_o = \frac{R_{an} - r}{r}\,[An]_i \qquad (9)$$

For bivalent cations the following equations should hold

$$\text{protein bound bivalent cation} = \Delta \text{ Cat} + (1 - r^2)[\text{Cat}]_o \quad (10)$$

and

$$[\text{Cat Pr}] = \frac{r^2 - R_{cat}}{r^2 \times R_{cat}}[\text{Cat}]_o = \frac{r^2 - R_{cat}}{r^2}[\text{Cat}]_i \quad (11)$$

SOME FACTORS INFLUENCING THE DONNAN RATIO

The influence of protein concentration and the concentration of filtrable ions on the Donnan ratio

Equation (6) shows quite clearly that the magnitude of r depends on the relative concentrations of filtrable ions and protein An increase in electrolyte concentration while protein concentration remains constant will increase r an increase in protein concentration while electrolyte concentration remains constant will decrease r Fig 3 shows

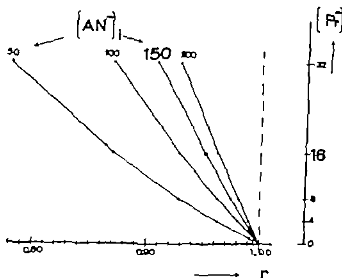


Fig. 3 Theoretical correlation between and negative net charge for different concentrations of An

the theoretical relationship between r and protein concentration at four different concentrations of filtrable ion It can be seen that at the anion concentration to be expected in human serum the relation between protein concentration and r is nearly linear Fig. 3 may serve to emphasise the need for details concerning protein and electrolyte concentration, when publishing values for the Donnan ratio Furthermore, since the net charge of the proteins changes with pH the latter must also be mentioned

The presence of protein on both sides of the membrane

In our discussion of the Donnan equilibrium we restricted ourselves on purpose to the conditions existing if a protein solution such as serum is dialysed *in vitro* against an electrolyte solution containing no protein. This was the situation in our *in vitro* experiments (to be described later) and to them the equations given in the previous paragraph should be applicable without modification *In reality however* the interstitial fluid contains protein both mobile and fixed (connective tissue). The situation at the capillary

membrane is therefore different from that depicted in fig. 2 since charged macromolecules are present on *both* sides of the membrane. The effect on the Donnan ratio will be to increase it, since the latter is determined by the *difference* between the non-filtrable charges present on both sides of the membrane. If the charges balance each other r will become 1.000. At the capillary membrane we meet with the difficulty that the magnitude of the non-filtrable charge on the side of the interstitial fluid is unknown (chapter IV). Although therefore an accurate prediction is impossible, the Donnan ratio existing at the capillary membrane must be nearer 1.000 than the Donnan ratio found in *in vitro* experiments, where plasma or serum are equilibrated against protein-free solutions.

REVIEW OF DATA CONCERNING THE INTERACTION BETWEEN ALBUMIN AND INORGANIC IONS

Albumin is the one plasma protein which has been obtained in sufficiently large quantities and in a sufficiently pure state to permit extensive *in vitro* studies to be made of its

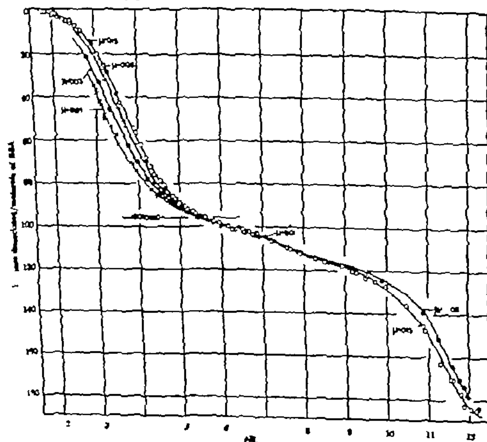


Fig. 4. H-ion titration curves of bovine serum albumin (at 25°C and at four ionic strengths). Reproduction of fig. 1 in publication of Tanford *et al.* (1955) Am. J. chem. Soc. 77, 6414.

interaction with filtrable ions Some of the results are of interest not only as an illustration of the interaction between filtrable ions and protein molecules in general but also because *they will aid us in the interpretation of the results* of our experiments with plasma and serum

INTERACTION BETWEEN ALBUMIN AND H^+-IONS

Titration curve – The effects of temperature, ionic strength and the presence of other cations

Fig. 4 shows the titration curve *obtained fo a crystalline preparation of bovine serum* albumin (TANFORD *et al* 1955) It will be seen that the total number of H^+-ions which *can be dissociated per molecule of albumin is of the* order of 200 Above pH 5.5 which is approximately the isoelectric pH the net charge is negative The value of the latter increases from about 8 to about 16 when the pH increases from 6 9 to 7 8 This demon*strates that albumin is but a poor buffer substance in the physiological pH range* Changes in ionic strength appear to have no influence on this part of the curve. Changes in temperature have some effect as is shown in fig. 5 It was taken from an earlier publication of TANFORD (1950) and represents results obtained with a somewhat different

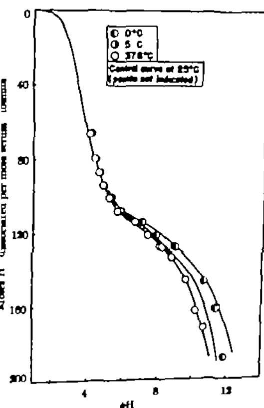


Fig. 5 – H ion titration curves of human serum albumin (mercaptalbumin) t different temperatures (ionic strength 0 15). Reproduction of fig 3 in publication of TANFORD (1950) Am. J chem. Soc. 72 441

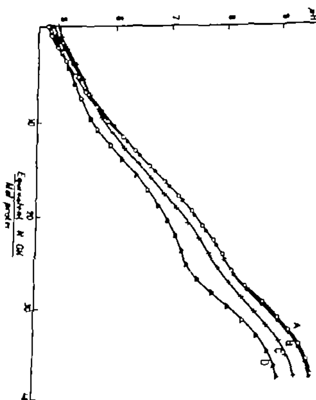


Fig. 6 H -ion titration curves of bovine serum lbumin (at 25°C and t four different electrolyt concentr ions). Reproduction of the figure in the public tion of KATES (1954) C. R Acad. Sc (Paris) 239' 117 The original figure ha been rotated ninety degrees clockwise for reasons explained in the text.

preparation of human serum albumin The influence of the presence of other cations on the titration curve is demonstrated in fig. 6 It was taken from a publication by KEPES (1954) and has been turned 90 degrees in clockwise direction in order to make ordinate and abcis comparable with those of the curves in fig. 4 and 5 Figure 6 then shows four titration curves of one non-crystalline preparation of bovine serum albumin

Curve A was obtained when no salt was added to the albumin solution curve B when the ionic strength was very low (between 0.001 and 0.003, by adding very small amounts of KCl) curve C when sufficient KCl was added to make the ionic strength 0.029 and curve D when sufficient $CaCl_2$ was added to bring the ionic strength also to a value of 0.029 In each case the protein concentration was 4 g/L

From fig. 6 it is evident that in the physiological pH range the type of ion present in the solution has a definite effect on the slope of the titration curve of albumin Fig. 6 also shows that with decreasing H -ion concentration the other cations can compete more effectively with the H -ions for the dissociated groups on the protein molecules. This is especially so for the bivalent cations as is demonstrated by the fact that, when $CaCl_2$ was added instead of KCl the number of H -ions dissociated per mole of albumin at pH 7 4 increased by about 5

INTERACTION BETWEEN ALBUMIN AND CATIONS OTHER THAN H^+-IONS

Competition between H -ions and other cations

When fully dissociated an albumin molecule has some 200 binding sites for H -ions. They are not of equal strength and by ingenious analysis of titration data, TANFORD *et al* (1955) distinguished the following ionogenic groups as binding sites for H -ions (in order of increasing strength of *association* the numbers between brackets indicate groups per mole of albumin) β- γ-carboxyl (99) imidazole (16), α-amino (1), ε-amino (57), phenolic (19), guanidine (22) and sulfhydryl (1). In principle these sites should be available to all cations, but the H -ion has such advantages that in general the other cations can only compete if their concentration is considerably higher than that of the H -ions This is reflected by the fact that the binding of other cations increases when the H -ion concentration decreases. The metal ions best suited to compete with the H -ions instructive examples of this competition are given by TANFORD (1952) – are those of the intermediate series in the Periodic System (Cu^{2} Zn^{2} Fe^{2} Co^{2+} a.o.) This high affinity is explained by a typical electron structure, but probably because of this very characteristic the number of binding sites for which these ions can compete effectively with H -ions is limited. For details the reader may be referred to GURD and WILCOX' lucid and instructive review (1956). An expression of the specialised character of this complex type of cation binding is the fact that it can occur at a pH value where the net charge of the protein is zero or even positive

For the metal ions mentioned the tendency to become attached by complex binding is so strong that their interaction with proteins can be considered to be solely of this type This is probably not so for the alkaline earth metal ions and certainly not so for the univalent alkali metal ions

Interaction with Ca^{2+} and Mg^{2+}

These bivalent cations are partially complex bound presumably at ionogenic groups which provide an opportunity for chelation There is however evidence that in the physiological pH range some of the Ca^{2+} ions are not bound in true complex-compounds, but attracted by electrostatic forces of sufficient strength to decrease the activity coefficient of these Ca^{2+} ions below the value calculated from the ionic strength of the solution (see for instance GURD & WILCOX 1956) For reasons discussed already association constants calculated for Ca^{2+} binding by proteins have therefore a somewhat dubious value.

Interaction with Na^{+} and K^{+}

These univalent cations are apparently bound by albumin in a salt type manner only up to pH values as high as 8 0 (SCATCHARD *et al* 1950[1] CARR 1956 VAN OS & KOOPMAN VAN EUPEN 1957) But neither Scatchard s experiments nor those of the Dutch workers can exclude the possibility that at physiological pH values up to 5 / of the Na^{+} and K^{+} present in solution are bound in a complex type of manner

The experiments of Scatchard *et al* were done at a pH value near the IEP and they involved subtraction of results obtained by different methods at different temperatures. Van Os and Koopman-Van Eupen did their experiments at a pH of about 7 70 but in some of the experiments the conductance of Na and K-albuminate was found to be 5–10 lower than that of NaCl and KJ

CARR used a special technique with measurement of membrane potentials and in his experiments the margin of uncertainty appears to be smaller However the concentration of cation was rather low (5-20 mM/L) in comparison to the protein concentration (about 30 g/L) Other experiments suggest that the amount of complex bound ion depends on the concentration of the particular ion (see for instance for Ca^{2} CARR 1953[1]) and Carr s results do not therefore exclude the possibility that at physiological concentrations a small amount of Na^{+} (1–2 /) is bound by albumin in a complex type of manner It should moreover be mentioned that Carr found a definite decrease in Na^{+} and K^{+} activity in the presence of (non purified) α and β-globulins and fibrinogen

The results given in Table IV of CARR's publication (1956) indicate that at pH 7.5 and in the presence of about 5–20 mEq/L cation, 2 3 mEq of Na are complex-bound per 100 g of α and β-globulins. Expressed as a percentage of total cation concentration, complex-bound Na was of the order of 10–20 %.

Of particular interest are the experiments of DORIMUS & JOHNSON (1958), who used measurements of conductance and transference to study the interaction of Na and Ca^{2+} with (reasonably purified) bovine serum albumin They considered that a small ion is bound to a protein ion when the former migrates with the protein ion under the conditions of the electrolysis experiment This experimental approach is apt to give a fairly good indication of the total amount of ion attracted both by electrostatic forces and by complex binding. Their results indicate that Na is without doubt bound by albumin (10 ions per molecule at a net negative charge of about 25), but the authors concluded that the sodium ions form only weak ion pairs with the protein ion charges or even that the small ions are merely held in the charged sphere of the whole protein ion This situation is best explained by pure electrostatic interaction and in the summary they state that the appreciable sodium binding found was primarily electrostatic

The fact that the results of Dorsman and Johnson are sometimes quoted as evidence for complex binding of Na by albumin is an example of the misunderstandings which arise from the use of the term 'binding' in more than one sense.

We can conclude that the experimental evidence suggests that albumin binds the alkali metal ions in a salt-type manner only but that it cannot exclude the possibility that up to 2% of the Na and K present in an albumin solution is bound in a complex-type manner

INTERACTION BETWEEN ALBUMIN AND ANIONS

Albumin is rather remarkable in its affinity for inorganic and organic anions at pH values well above its IEP as GROLLMAN (1925) was the first to demonstrate.

Interaction with Cl

In the context of this study the most interesting interaction is that with Cl which was detected by Scatchard *et al* in consequence of the unexpected deviation in osmotic pressure of an albumin solution in 0.15 molar NaCl solution (SCATCHARD *et al.*, 1944). Subsequent studies revealed the fact that at pH values near the IEP Cl -activity decreased considerably when albumin was added (SCATCHARD *et al.*, 1950[1]) and thiocyanate activity even more so (SCATCHARD *et al.*, 1950[2]). We have been unable however to find in the articles published by Scatchard *et al* data on Cl binding at physiological pH with two exceptions.

Firstly in their original publication in the *Journal of Clinical Investigation* (SCATCHARD *et al.*, 1944) and in two following papers (SCATCHARD 1949 and SCATCHARD *et al.* 1946) the deviation from the expected osmotic behaviour subsequently proved to be due to complex-type binding of Cl^- appears to be very slight at pH 7.00 and insignificant at pH 7.40.

Secondly SCATCHARD *et al.* (1957), reporting experiments with exchange-electrodes, give in Table IV of their publication the following three values for moles Cl bound per mole albumin at pH values above 5.5 1.69 at pH 7.24 0.27 at pH 8.08 and −2.71 at pH 10.31 The values refer to solutions containing ± 10 g/L bovine serum mercapt-albumin and ± 10 mM/L of NaCl and NaOH each. With increasing concentration of NaCl but constant protein concentration the amount of protein-bound Cl increases. From experiments with serum albumin and NaCl at pH ± 5.0 (SCATCHARD *et al.*, 1950[1]) it would appear that an increase in Cl concentration from ± 7 to 140 mEq/L at an albumin concentration of 70 g/L doubles the amount of protein-bound Cl^-. Assuming a similar factor to apply in the higher *pH* range, Scatchard's observation at pH 7.24 ought be interpreted to indicate Cl binding of about 3 moles per mole albumin at pH ± 7.20. That would be equal to about 2 mEq of Cl per 40 g albumin the latter being the amount present in one litre of normal serum.

Other authors have extended their measurements to include a pH value of 7.0 ALBERTY & MARTIN (1951) using bovine serum albumin found (at pH 7.0, temp 0°C, albumin concentration 8 g/L and NaCl 0.15 mole/L) the rather high value of 7.6 mole Cl /mole albumin CARR (1953[2]) studied a number of proteins at pH values ranging from 3.0 to 7.0 His data are well suited for estimating the amount of protein-bound Cl at physiological pH

In the first place his results indicate that at pH higher than 5.5 serum globulins bind no Cl^- in complex-type manner But for our present discussion the most important data are to be found in his graphical presentation (fig. 2 in the 1953[2] publication) of moles of Cl bound per 10^5 g protein over the pH range 3.0 to 7.0 From this graph it would appear that at Cl concentration

of 100–120 mEq/L the following amounts are bound by serum albumin (moles Cl^- per 10^5 g protein). At pH 5.0 10.6, at pH 5.5 6.9 at pH 6.0 4.2, at pH 6.5 2.6 and at pH 7.0 1.7. By extrapolation we find a value of 1.4 at pH 7.4. At this pH then 40 g albumin would bind about 0.6 mEq Cl.

The evidence suggests that some Cl^- is complex bound by albumin at physiological pH but the amount is somewhat dubious. Scatchard's own data and those of Carr indicate that at pH 7.40 a value between 0.5 and 3.0 mole Cl per mole albumin is the most reasonable estimate. It must be stressed that this refers to purified preparations of albumin in solutions containing no bivalent cations.

Interaction with HCO_3^-

Concerning the interaction between HCO_3^- and albumin very little is known. KLOTZ & URQUHART (1949) observed that albumin shows a slight decrease in its affinity for methylorange in the presence of bicarbonate ions (concentration of the latter 100 mEq/L). Presumably HCO_3^- competes with methylorange for binding sites on the albumin molecules. In the experiments of Klotz and Urquhart the pH was 8.60 and it is conceivable that the observed effect would have been more pronounced at pH values around 7.40. But quantitative data on HCO_3^--binding are not available.

THE MEASUREMENT IN PLASMA (OR SERUM) OF PROTEIN BOUND ION. NET PROTEIN BOUND CATION (NCE) AND OF PROTEIN NET CHARGE

THE IMPORTANCE OF STUDYING PLASMA OR SERUM

When we aim at understanding phenomena such as the ion equilibrium at the capillary wall and the NCE and net charge of the plasma proteins it is necessary to study ion protein interaction in plasma or at least in serum. Extrapolation from results obtained with purified protein fractions to the conditions existing in plasma is not without danger in the case of ions such as Na^+, K^+, Cl, HCO_3, Ca^{2+} and Mg^2 which are to a very large or a large extent bound in a salt type manner. In the case of the ions that are almost exclusively complex bound (Cu^{2+}, Fe^{2+}, Mn^{2+}, Zn^{2+} etc.) the results of experiments with purified proteins are in general applicable to plasma because true complex binding is relatively insensitive to changes in pH, in ionic strength and in the nature of the other ions present in solution. But the salt type of binding is much more sensitive and in its case conclusions reached from observations on relatively simple solutions are not necessarily valid for plasma.

ASSUMPTIONS MADE WHEN STUDYING ION PROTEIN BINDING IN PLASMA OR SERUM

In order to interpret ion ion interaction in a simple solution of strong electrolytes a number of simplifying assumptions has to be made. Their number increases when ion binding by purified proteins is studied and when ion protein binding is analysed in solutions such as plasma the simplifications involved become considerable. The most important which we will have to make are

(1) In as far as NCE and protein net charge are concerned plasma is essentially an aqueous solution containing H Na K Ca^{2} Mg^{2} Cl HCO_3 and protein

The contribution of other cations present in plasma is negligible under *in vivo* conditions, but this is not so for the other anions (chapter II, IV and V).

(2) Plasma has the same ionic strength as interstitial fluid.

The consequences of this assumption have already been discussed.

(3) Protein is the only non-filtrable substance in plasma.

(4) The plasma proteins can be treated as if they constitute one protein.

The last two assumptions determine the meaning of the term "protein" as used in our studies of plasma or serum. In order to be able to discuss this meaning we will first briefly describe the manner in which we have determined ion-protein binding and in what way the values obtained are expressed.

DETERMINATION OF PROTEIN-BOUND ION IN THE EXPERIMENTS TO BE REPORTED AND EXPRESSION OF THE VALUES OBTAINED

Essentially we have determined ion-protein binding by comparing solutions of equal ionic composition but with different concentrations of protein. Observed differences between the concentrations of the filtrable ions were correlated with the concomitant differences between the concentrations of protein. The concentration of the filtrable ions is expressed in mEq per Kg H_2O, that of protein in g per Kg H_2O. Protein-bound ion can therefore be calculated in mEq per Kg solvent water or in mEq per unit weight of protein. For the latter we have chosen 100 g, since plasma protein cannot be expressed in moles. In the same way the difference between the sum concentration of the filtrable cations and the sum concentration of the filtrable anions in a sample can be correlated to the protein concentration in order to obtain NCE. The latter is then again calculated either in mEq per Kg solvent water or in mEq per 100 g of protein.

Samples in which the protein concentration was presumably the only independent variable were obtained by means of the two methods already mentioned in the General Introduction, i.e. *in vivo* ultrafiltration (chapter IV) and equilibrium dialysis *in vitro* (chapter V).

THE MEANING OF THE TERM "PROTEIN" IN THE STUDIES TO BE REPORTED

With both methods one actually observes the effect not of protein per se but of all non-filtrable substances present in plasma. Since the observed effect is correlated with the total protein concentration as determined by the biuret method, we do in fact correlate *the amount of filtrable ion bound by all non-filtrable plasma substances with the amount of plasma protein*. Since the latter is not necessarily the only non-filtrable substance present in plasma (which also contains for instance polysaccharides) an unknown error is introduced. In normal plasma this error is presumably negligible, but in pathological plasma it may be significant. This must be kept in mind when the results obtained with plasma are compared with those obtained with purified protein fractions.

Furthermore the proteins in plasma are by no means pure They have associated with varying amounts of inorganic ions, with saccharides, with steroids, with fatty acids etc. Indeed the biological importance of the plasma proteins is to a large extent determined by their ability to bind substances in a reversible manner The latter characteristic was stressed years ago by BENNHOLD (1932) and described as Vehikelfunktion More recently KLOTZ and co-workers have demonstrated that this tendency exists towards numerous inorganic and organic ions They remark (KLOTZ & URQUHART 1949) that the ion protein complexes thus formed may differ appreciably in physicochemical properties from those to be anticipated for the simple protein molecule (for which we can read the purified protein traction) It is therefore quite possible that the same plasma protein will be found to behave differently towards the inorganic ions, according to whether normal plasma is studied or for instance, uraemic or hyperlipaemic plasma

Finally when for any given plasma the concentration of the filtrable ions is correlated with the total protein concentration it is tacitly assumed that the relative contribution of the various plasma proteins to the total protein concentration is the same in the samples that are compared In chapter IV the correctness of this assumption in the case of the methods used will be tested When results obtained for different plasmas are compared it must be realised that a difference in the amount of ion bound per 100 g of protein may be due to either a difference in the composition of (total) protein or to an abnormally low or high ion binding by one or more of the plasma proteins. Judicious comparison of the results obtained for plasma samples with different protein composition may then provide an answer

THE DETERMINATION OF PROTEIN NET CHARGE IN PLASMA

For the purpose of this study net charge was defined as the difference between the equivalent concentrations of dissociated filtrable cations and dissociated filtrable anions According to this definition the magnitude of net charge is equal to the difference between the amounts of cations and of anions that are bound in a salt-type manner and it is expressed in mEq per Kg solvent water or mEq per 100 g of protein This indirect definition is necessary because, in contrast to solutions containing one well defined protein, one cannot in the case of plasma calculate net charge from the titration curve of the protein and the dissociation constants of its acid groups. Moreover the number of purified proteins of which the titration curve is accurately known is very small Among these is serum albumin but it remains to be seen whether the titration curve is the same for albumin such as it is in plasma It is impossible to obtain a complete titration curve for plasma because of the instability of many globulins. Titration of plasma over the physiological pH range gives information concerning the overall buffer capacity for that pH range but the presence of weak acids and bases other than protein prevents the interpretation of such titration data in terms of protein net charge

Although we cannot determine the concentrations of dissociated cations and anions in plasma the above mentioned definition of protein net charge has the advantage of relating it to NCE and to the amounts of complex bound cation and anion (see page 17) Since NCE can be determined and presumably the amounts of complex bound cation and anion also (see page 23) a value should be obtainable for net charge But

it has already been pointed out that this value is not easily interpreted in terms of a definite number of charged groups on the protein molecules.

SOME OF THE RELATIONS POSSIBLE BETWEEN THE NCE AND THE NEGATIVE NET CHARGE OF THE PROTEINS IN PLASMA

Fig. 7 is an elaborate version of fig. 2 and it shows some of the relations possible in the physiological pH range. Four different types of protein molecules are compared. They

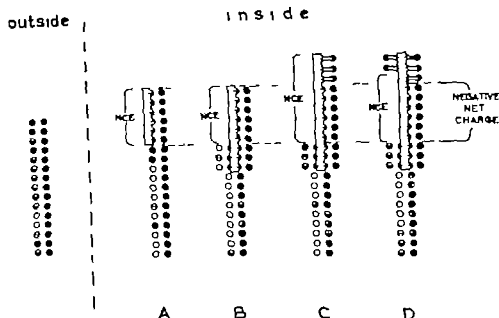


Fig. 7 Schematic representation of possible relationships between NCE and negative net charge of the proteins in plasma (physiological pH range).

all have the same negative net charge, but they differ in regard to the concomitant presence of a positive charge on the protein and in regard to their tendency to bind filtrable cations and anions in a complex-type manner. All four protein solutions are considered to be in equilibrium through a semipermeable membrane with the same electrolyte solution. Since the four proteins carry the same negative net charge and since the concentration of filtrable dissociated anions in the inside solution is the same in every case, the Donnan ratio is also the same for each of the four equilibria.

Equilibrium A represents the most simple possibility. The protein molecule carries a negative charge only and there is no complex-type of binding. NCE is therefore equal to the total negative charge on the protein, which in this case is also the net charge. Furthermore the concentration ratios of the cations and the anions are identical with the Donnan ratio.

Equilibrium B represents a protein molecule which carries both negative and positive charges but more negative ones. There is no complex type of binding. NCE is again

equal to the negative net charge, but the latter is smaller than the *total negative charge*. The concentration ratios of cations and anions are still identical with the Donnan ratio

Equilibrium C The protein carries now both negative and positive charges but in addition some cation is complex-bound NCE exceeds therefore the negative net charge by an amount equal to the equivalents of cation that are bound in a complex type manner Whereas the concentration ratio of the anions is still identical with the Donnan ratio the concentration ratio of the cations is smaller

Equilibrium D In addition to carrying both negative and positive charges and binding some cation in a complex type of manner the protein now also binds some anion in this manner (in our example less anion than cation) In this case NCE is again larger than the negative net charge but the difference is less than in the case of C since the complex bound anion partially balances the complex bound cation. For both the cations and the anions the concentration ratio now differs from the Donnan ratio but whereas the concentration ratio will be smaller in the case of the cations it will be larger in the case of the anions

According to the data obtained with purified protein fractions it would appear that the situation described under D is the most likely one to be encountered in normal plasma.



Summary of chapter I

For the purpose of the present study plasma is considered an aqueous solution containing filtrable inorganic ions (H^+ Na^+ K^+ Ca^{2+} Mg^{2+} Cl^- HCO_3^-) and non filtrable organic ions (protein)

The various types of interaction between the inorganic ions and the proteins are discussed, and binding is defined as attraction of ions by protein regardless of the nature and the strength of the attracting forces For practical purposes binding is distinguished in two types according to its effect on the activity coefficient of the ions. When the activity coefficient is not different from that found in a solution of equal ionic strength and composition but containing no protein, the binding is considered to be of the salt type, *i.e.* the protein-bound ion behaves as if fully dissociated. When however the activity coefficient decreases in the presence of protein the binding is considered to be of the complex-type *i.e.* protein bound ion behaves as if it is no longer dissociated The difference between the two types of binding becomes manifest when solutions such as plasma are subjected to equilibrium dialysis As the Donnan equilibrium concerns active ions only the presence of ion bound in a complex type manner will cause the concentration ratio of the particular ion to differ from the Donnan ratio

Plasma contains many different ions and proteins, the latter carrying both positive and negative charges. A measure of the overall effect of ion protein binding is the difference between the sum concentration of the filtrable cations and the sum concen

tration of the filtrable anions. This net cation equivalency or NCE (old term base binding power') is an empirical quantity and should not be mistaken for the negative net charge of the proteins. The latter is equal to the difference between the amounts of cation and anion bound in a salt type manner whereas NCE is equal to the difference between the total amounts of protein-bound cation and anion and includes therefore the ions bound in complex-type manner

The pragmatic nature of the definitions given is stressed

Review of literature concerning the net cation equivalency of plasma proteins the normal concentration of the main filtrable ions in plasma and the distribution of the latter between plasma and interstitial fluid

This review is mainly devoted to data concerning NCE of the plasma proteins and data concerning the interaction between the latter and the *uni*valent ions For recent reviews concerning the interaction between Ca and the plasma proteins the reader is referred to TORIBARA *et al* (1957) to TEREPKA *et al* (1958) to PRASAD (1960) and to WALSER (1961 1962) For Mg we refer to HASSELMAN & VAN KAMPEN (1958) to PRASAD (1961) and to WALSER (1961)

Leaving out the alkaline earth metal ions considerably reduces the amount of literature to be reviewed But even so it is impossible to be complete, especially where the older literature is concerned Only those publications will be cited which provided new data, presented a new method or contributed in any other way materially to the elucidation of the problem under discussion The literature from the previous century often makes fascinating reading because of the thoughts which were already developed then and the often painstaking documentation of the experimental results. As an example we should like to mention the work of SCHMIDT (1850) who was apparently the first to determine electrolyte concentrations in human serum and the work of LOEWY & ZUNTZ (1894) who as far as we know made the first attempt to determine die Bindung der Alkalien in Serum From dialysis experiments with horse serum they concluded that *ein Theil des Alkalis derart gebunden ist dass es am Diffusionsprozesse nicht theilnehmen kann*

NCE OF PLASMA PROTEINS (NORMAL PLASMA)

According to its definition NCE is obtained by subtracting the sum concentration of the filtrable anions from the sum concentration of the filtrable cations However the accurate determination of both these concentrations is no easy matter In fact only Van Slyke and co-workers Darrow & Hartmann and Henderson *et al* have attempted to determine the value of NCE accurately To this end they first removed all filtrable electrolytes from the plasma proteins and then redissolved the latter in a solution containing only Na Cl and HCO_3 ions A few authors have subsequently tried to verify the values found by the Van Slyke group by comparing these values with those obtained when for plasma or serum the sum of the concentrations of Cl HCO_3, inorganic phosphate and an assumed value for (SO_4 + organic acids) was subtracted from the sum concentration of Na, K Ca and Mg.

We will first present and discuss the work of VAN SLYKE and associates and that of DARROW & HARTMANN and of HENDERSON *et al* Later we will make an estimate of

NCE from the values reported for the normal plasma concentrations of Na, K, Ca, Mg, Cl, HCO_3 and inorganic phosphate

THE EXPERIMENTS OF VAN SLYKE AND CO WORKERS AND ADDITIONAL EXPERIMENTS DONE BY DARROW & HARTMANN AND BY HENDERSON AND CO WORKERS

As the value found by Van Slyke and co-workers is the one always quoted their experimental evidence must be discussed in some detail. They determined NCE in the following manner To a protein solution which had been freed from all electrolytes other than H-ions by prolonged dialysis against distilled water an accurately known amount of $NaHCO_3$ and NaCl (Na and Cl in physiological concentration) was added and the resulting solution equilibrated at 38°C with CO_2 at various pressures.

Water content was determined by drying at 110°C to constant weight, protein concentration by macro-Kjeldahl analyses, accepting a nitrogen factor of 6.25 total CO_2 by the manometric method P_{CO_2} by Haldane analyses of the gas

$$H_2CO_3 \text{ from } \frac{1000 \quad \text{co}_2}{22.26} \quad \frac{P_{CO_2}}{760} \text{ where } \quad \text{co}_2$$

had been determined in previous experiments HCO_3 from total $[CO_2]$ $[H_2CO_3]$ pH in most experiments by means of the hydrogen electrode at 38°C. Na and Cl concentration were calculated from the weighed amount of $NaHCO_3$ and NaCl added to the protein solution. In some cases as control total base was determined by the method of Fiske

As the solutions were considered to contain only one type of cation (Na), and three types of anion (Pr⁻ Cl and HCO_3) it was concluded that $[Na\ ] = [Pr^-] + [HCO_3^-]$ $- [Cl\ ]$ (H and OH can be neglected for quantitati e calculations). The possibility of the proteins attracting anions was therefore not considered and ion-protein binding was tacitly assumed to be of the salt-type only By subtracting the sum concentration of Cl and HCO_3 from the Na concentration of the solutions studied Van Slyke *et al* obtained the amount of 'base bound' by protein. In their equations this quantity was indicated by BP and from chapter I it will be evident that it is identical with NCE. From the protein content of the solution BP could then also be calculated in terms of mEq per unit weight of nitrogen or protein By determining BP at various values of pH, a relation between BP and pH was obtained. Thus Van Slyke and co-workers expressed as d BP BP dpH (that is to say increase in BP per unit increase in pH) and termed molecular buffer value

The method was first applied in the determination of the NCE of crystalline Hb and HbO_2 (VAN SLYKE *et al* 1922, HASTINGS *et al.* 1924). In their classic paper on the factors controlling the electrolyte and water distribution in blood, VAN SLYKE, WU & MCLEAN (1923) report for the first time experiments on the base binding power of serum proteins. Two samples of horse serum were used, which had been dialysed until less than 0 001 N electrolyte per litre remained. The protein concentration of the samples was 50.3 g/L and 47 2 g/L respecti ely the Na concentration 130.5 mEq/L. For each sample BP was determined at four pH values, varying from about 7 10 to 7 74 As a linear relation was found between pH and BP the following equation was calculated

Review of literature concerning the net cation equivalency of plasma proteins the normal concentration of the main filtrable ions in plasma and the distribution of the latter between plasma and interstitial fluid

This review is mainly devoted to data concerning NCE of the plasma proteins and data concerning the interaction between the latter and the *uni*valent ions. For recent reviews concerning the interaction between Ca and the plasma proteins the reader is referred to TORIBARA *et al* (1957) to TEREPKA *et al* (1958) to PRASAD (1960) and to WALSER (1961 1962) For Mg we refer to HASSELMAN & VAN KAMPEN (1958), to PRASAD (1961) and to WALSER (1961)

Leaving out the alkaline earth metal ions considerably reduces the amount of literature to be reviewed But even so it is impossible to be complete, especially where the older literature is concerned. Only those publications will be cited which provided new data, presented a new method or contributed in any other way materially to the elucidation of the problem under discussion The literature from the previous century often makes fascinating reading because of the thoughts which were already developed then and the often painstaking documentation of the experimental results As an example we should like to mention the work of SCHMIDT (1850) who was apparently the first to determine electrolyte concentrations in human serum and the work of LOEWY & ZUNTZ (1894) who as far as we know made the first attempt to determine die Bindung der Alkalien in Serum From dialysis experiments with horse serum they concluded that ein Theil des Alkalis derart gebunden ist dass es am Diffusionsprozesse nicht theilnehmen kann

NCE OF PLASMA PROTEINS (NORMAL PLASMA)

According to its definition NCE is obtained by subtracting the sum concentration of the filtrable anions from the sum concentration of the filtrable cations However the accurate determination of both these concentrations is no easy matter In fact only Van Slyke and co-workers Darrow & Hartmann and Henderson *et al* have attempted to determine the value of NCE accurately To this end they first removed all filtrable electrolytes from the plasma proteins and then redissolved the latter in a solution containing only Na, Cl and HCO_3 ions A few authors have subsequently tried to verify the values found by the Van Slyke group by comparing these values with those obtained when for plasma or serum the sum of the concentrations of Cl, HCO_3, inorganic phosphate and an assumed value for (SO_4 + organic acids) was subtracted from the sum concentration of Na, K Ca and Mg.

We will first present and discuss the work of VAN SLYKE and associates and that of DARROW & HARTMANN and of HENDERSON *et al* Later we will make an estimate of

In their publication the protein values were expressed in grams of N. To make the equation comparable with the older ones we have expressed it in terms of grams of protein assuming an N factor of 6.25, as this was the factor which Van Slyke used for calculating equation (D).

In normal human serum Van Slyke *et al* found with their fractionation technique the ratio albumin-globulin to be 1 6 From (C) they calculated therefore for normal human serum the equation

$$BP = 0\ 104\ P\ (pH - 5.08) \qquad (D)$$

According to (D) the base binding power' that is the NCE, of 100 g human serum protein (at pH 7.35 and 38°C) is 23 6 mEq and increases 10 4 mEq per unit pH

The correctness of extrapolating the values found with horse serum proteins to human serum was tested with one sample of pooled human serum which had been dialysed for three months at 0°C. It contained 17.2 g/Kg H_2O albumin and 14 4 g/Kg H_2O globulin The CO_2 titration was carried out at 4 different values of pH (6 01 to 7.97), the Na concentration was 136 mEq/Kg H_2O and the temperature 38°C. At every pH the BP found was 5–7 / lower than the value calculated from equation (D). At the end of their article Van Slyke *et al* commented on the fact that the earlier equations indicated a considerably lower value for BP They concluded that probably in the earlier experiments dialysis had not been pushed far enough to remove the last traces of pre-existing base from the protein preparations used.

Equation (C) and (D) have generally been accepted, and the value of 16 mEq/L inserted in the usual cation-anion diagrams for plasma or serum is based on them. Table I summarises the results obtained in the experiments of Van Slyke and co-workers. The NCE values were calculated for pH 7 35 and expressed in mEq per 100 g protein This was done because in our experiments to be discussed later all values have been expressed per 100 g protein The pH 7.35 was selected because generally not arterial but venous blood is analysed, which in the resting forearm appears to have a pH of on the average 7 35

It should be stressed that Van Slyke *et al.* did their experiments at 38°C. The observations of S AUNt *et al.* (1925) on the effect of temperature on acid-base-protein equilibrium in the pH range 7 00–7 70 suggest that whereas the molecular buffer value of the plasma proteins is not affected when the temperature changes from 38°C to 20°C, the apparent IEP increases by 0 02 on the average for each centigrade decrease in temperature. Accordingly the value of NCE should be about 3.3 mEq/100 g protein lower at 20°C than at 38°C, other conditions remaining equal.

In view of the accuracy needed the experiments of Van Slyke are not easily repeated and it is therefore not surprising to find that only two other attempts have been made to determine the NCE of the plasma proteins.

In 1929 DARROW & HARTMANN pointed out that equation (B) of Van Slyke and co-workers could not be used to calculate 'base binding power in patients in whom the A/G ratio was abnormal. They did therefore some experiments with beef serum which had been dialysed for 2 to 3 days against distilled water in an atmosphere of CO_2 and in the cold During the dialysis part of the globulins precipitated.

After dialysis CO_2 titration after the manner of Van Slyke *et al.* was carried out separately on whole serum, on the washed precipitated globulins and on the proteins not precipitated by dialysis. Total protein was determined by the macro-Kjeldahl method using factor of 6.25 Albumin and globulin were determined in the same way after fractionation by 22.2 per cent disodium sulfate. Apparently two samples were studied, both at two pH values (about 7.2 and about 7.5).

(for the pH range covered by the experiments)

$$BP = 0.068\ P_s\ (pH - 4.80) \qquad (A)$$

where BP represents base bound by protein (i.e. NCE) in mEq and P_s serum protein in grams (The pH at which BP would be zero according to the equation – in this case 4.80 – will be indicated as 'apparent IEP') According to equation (A) the NCE of 100 g of normal horse protein is 17.4 mEq at pH 7.35 and increases by 6.8 mEq for every 1.0 unit increase in pH It is interesting to note that the values used in the general equations from which the authors calculated the Donnan ratios across the erythrocyte membrane and across the capillary wall are based on this equation

In 1927 in a paper concerned with the distribution of electrolytes between transsudates and serum, HASTINGS et al (1927) pointed out that equation (A) correlated BP with total protein only and that – as the titration curves of albumin and globulin differed greatly and moreover the albumin/globulin ratio was not the same in man as in the horse – the equation could not be used to calculate the base bound by human serum proteins They mentioned experiments in which the method described above was applied to dialysed human serum From these experiments the following relation was calculated for human serum

$$BP = 0.097\ P\ (pH - 5.26) \qquad (B)$$

Equation (B) indicates a NCE of 20.3 mEq per 100 g of (normally constituted) human serum at a pH of 7.35 increasing with 9.7 mEq for every 1.0 pH unit increase. The experimental data from which equation (B) was derived have never been published as far as we could ascertain

Instead, another equation was proposed in 1928 by VAN SLYKE et al It is based on the results obtained with albumin and globulin fractions of horse serum. The globulin was salted out from serum with half saturated $(NH_4)_2SO_4$, albumin with saturated $(NH_4)_2SO_4$ and both fractions were freed from small electrolytes by pressure dialysis against distilled water which had been saturated with CO_2 to bring its reaction near to the isoelectric point of the protein fractions In this study the protein solutions were not only titrated with CO_2, but with HCl also For that purpose the protein fractions were dissolved in enough NaOH to render the solution distinctly alkaline, and then titrated electrometrically with 0.1 N HCl The amount of cation opposed by protein valencies was found by subtracting the HCl added from the NaOH originally present in the solution

In three experiments 2 preparations of albumin (protein concentration about 50 g/Kg H_2O) were studied, and in another three experiments 2 preparations of globulin (protein concentration 70.9 and 36.2 g/Kg H_2O). The Na concentration was between 135 and 139 mEq/Kg H_2O and all experiments were done at 38°C Determinations were done at 3 to 7 different pH values, ranging from 5.20 to 9.26, but in the final analysis the authors have only included the values obtained at pH values between 6.50 and 7.80 In the calculation of HCO_3^- a value of 6.11 was assumed for pK_1' of carbonic acid.

The relation between pH and BP was again a linear one for both protein fractions and no difference was found between the results obtained with CO_2 and with HCl titration From their results the following equation was calculated for horse serum

$$BP = 0.124\ Alb\ (pH - 5.16) + 0.077\ Glob\ (pH - 4.89) \qquad (C)$$

In their publication the protein values were expressed in grams of N. To make the equation comparable with the older ones we have expressed it in terms of grams of protein assuming an N factor of 6.25, as this was the factor which Van Slyke used for calculating equation (D).

In normal human serum Van Slyke *et al.* found with their fractionation technique the ratio albumin-globulin to be 1.6. From (C) they calculated therefore for normal human serum the equation

$$BP = 0.104\ P\ (pH - 5.08) \qquad (D)$$

According to (D) the 'base binding power' that is the NCE, of 100 g human serum protein (at pH 7.35 and 38°C) is 23.6 mEq and increases 10.4 mEq per unit pH.

The correctness of extrapolating the values found with horse serum proteins to human serum was tested with one sample of pooled human serum which had been dialysed for three months at 0°C. It contained 17.2 g/Kg H_2O albumin and 14.4 g/Kg H_2O globulin. The CO_2 titration was carried out at 4 different values of pH (6.01 to 7.97), the Na concentration was 136 mEq/Kg H_2O and the temperature 38°C. At every pH the BP found was 5-7 % lower than the value calculated from equation (D). At the end of their article Van Slyke *et al.* commented on the fact that the earlier equations indicated a considerably lower value for BP. They concluded that probably in the earlier experiments dialysis had not been pushed far enough to remove the last traces of pre-existing base from the protein preparations used.

Equation (C) and (D) have generally been accepted and the value of 16 mEq/L inserted in the usual cation-anion diagrams for plasma or serum is based on them. Table I summarises the results obtained in the experiments of Van Slyke and co-workers. The NCE values were calculated for pH 7.35 and expressed in mEq per 100 g protein. This was done because in our experiments to be discussed later all values have been expressed per 100 g protein. The pH 7.35 was selected because generally not arterial but venous blood is analysed which in the resting forearm appears to have a pH of on the average 7.35.

It should be stressed that Van Slyke *et al.* did their experiments at 38°C. The observations of Stadie *et al.* (1925) on the effect of temperature on acid-base-protein equilibrium in the pH range 7.00–7.70 suggest that whereas the molecular buffer value of the plasma proteins is not affected when the temperature changes from 38°C to 20°C, the apparent IEP increases by 0.02 on the average for each centigrade decrease in temperature. Accordingly the value of NCE should be about 3.5 mEq/100 g protein lower at 20°C than at 38°C, other conditions remaining equal.

In view of the accuracy needed the experiments of Van Slyke are not easily repeated and it is therefore not surprising to find that only two other attempts have been made to determine the NCE of the plasma proteins.

In 1929 Darrow & Hartmann pointed out that equation (B) of Van Slyke and co-workers could not be used to calculate base binding power in patients in whom the A/G ratio was abnormal. They did therefore some experiments with beef serum which had been dialysed for 2 to 3 days against distilled water in an atmosphere of CO_2 and in the cold. During the dialysis part of the globulins precipitated.

After dialysis CO_2 titration after the manner of Van Slyke *et al.* was carried out separately on whole serum, on the washed precipitated globulins and on the proteins not precipitated by dialysis. Total protein was determined by the macro-Kjeldahl method using a factor of 6.25. Albumin and globulin were determined in the same way after fractionation by 22.2 per cent disodium sulfate. Apparently two samples were studied, both at two pH values (about 7.2 and about 7.5).

(for the pH range covered by the experiments)

$$BP = 0.068\ P\ (pH - 4.80) \tag{A}$$

where BP represents base bound by protein (*i e* NCE) in mEq and P_s serum protein in grams (The pH at which BP would be zero according to the equation – in this case 4 80 – will be indicated as apparent IEP") According to equation (A) the NCE of 100 g of normal horse protein is 17 4 mEq at pH 7 35 and increases by 6 8 mEq for every 1.0 unit increase in pH It is interesting to note that the values used in the general equations from which the authors calculated the Donnan ratios across the erythrocyte membrane and across the capillary wall are based on this equation

In 1927 in a paper concerned with the distribution of electrolytes between transudates and serum HASTINGS *et al* (1927) pointed out that equation (A) correlated BP with total protein only and that – as the titration curves of albumin and globulin differed greatly and moreover the albumin/globulin ratio was not the same in man as in the horse – the equation could not be used to calculate the base bound" by human serum proteins. They mentioned experiments in which the method described above was applied to dialysed human serum From these experiments the following relation was calculated for human serum

$$BP = 0.097\ P_s\ (pH - 5.26) \tag{B}$$

Equation (B) indicates a NCE of 20 3 mEq per 100 g of (normally constituted) human serum at a pH of 7 35 increasing with 9 7 mEq for every 1 0 pH unit increase. The experimental data from which equation (B) was derived have never been published as far as we could ascertain

Instead another equation was proposed in 1928 by VAN SLYKE *et al* It is based on the results obtained with albumin and globulin fractions of horse serum The globulin was salted out from serum with half saturated $(NH_4)_2SO_4$ albumin with saturated $(NH_4)_2SO_4$ and both fractions were freed from small electrolytes by pressure dialysis against distilled water which had been saturated with CO_2 to bring its reaction near to the isoelectric point of the protein fractions In this study the protein solutions were not only titrated with CO_2, but with HCl also For that purpose the protein fractions were dissolved in enough NaOH to render the solution distinctly alkaline and then titrated electrometrically with 0 1 N HCl The amount of cation opposed by protein valencies was found by subtracting the HCl added from the NaOH originally present in the solution

In three experiments 2 preparations of albumin (protein concentration about 50 g/Kg H_2O) were studied, and in another three experiments 2 preparations of globuli (protein concentration 70.9 and 36.2 g/Kg H_2O). The Na concentration was between 135 and 139 mEq/Kg H_2O and all experiments were done at 38°C. Determinations were done at 3 to 7 different pH values, ranging from 5.20 to 9.26, but i the final analysis the authors ha e only included the values obtained at pH values between 6.50 and 7.80. In the calculation of HCO_3 a value of 6.11 was assumed for pK of carbonic acid.

The relation between pH and BP was again a linear one for both protein fractions and no difference was found between the results obtained with CO_2 and with HCl titration From their results the following equation was calculated for horse serum

$$BP = 0.124\ Alb\ (pH - 5.16) + 0.077\ Glob\ (pH - 4.89) \tag{C}$$

In their publication the protein values were expressed in grams of N To make the equation comparable with the older ones we have expressed it in terms of grams of protein assuming an N factor of 6 25 as this was the factor which Van Slyke used for calculating equation (D).

In normal human serum Van Slyke *et al* found with their fractionation technique the ratio albumin-globulin to be 1 6 From (C) they calculated therefore for normal human serum the equation

$$BP = 0\ 104\ P\ (pH - 5.08) \qquad (D)$$

According to (D) the 'base binding power' that is the NCE, of 100 g human serum protein (at pH 7.35 and 38°C) is 23.6 mEq and increases 10.4 mEq per unit pH

The correctness of extrapolating' the values found with horse serum proteins to human serum was tested with one sample of pooled human serum which had been dialysed for three months at 0 C. It contained 17.2 g/Kg H_2O albumin and 14 4 g/Kg H_2O globulin. The CO_2 titration was carried out at 4 different values of pH (6.01 to 7 97), the Na concentration was 136 mEq/Kg H_2O and the temperature 38°C. At every pH the BP found was 5–7 / lower than the value calculated from equation (D). At the end of their article Van Slyke *et al* commented on the fact that the earlier equations indicated a considerably lower value for BP They concluded that probably in the earlier experiments dialysis had not been pushed far enough to remove the last traces of pre-existing base from the protein preparations used

Equation (C) and (D) have generally been accepted and the value of 16 mEq/L inserted in the usual cation-anion diagrams for plasma or serum is based on them Table 1 summarises the results obtained in the experiments of Van Slyke and co-workers. The NCE values were calculated for pH 7 35 and expressed in mEq per 100 g protein. This was done because in our experiments to be discussed later all values have been expressed per 100 g protein. The pH 7.35 was selected because generally not arterial but venous blood is analysed which in the resting forearm appears to have a pH of on the average 7.35

It should be stressed that Van Slyke *et al.* did their experiments at 38°C. The observations of 8 Adair *et al.* (1925) on the effect of temperature on acid-base-protein equilibrium' in the pH range 7 00–7 70 suggest that whereas the molecular buffer value of the plasma proteins is not affected when the temperature changes from 38°C to 20°C, the apparent IEP increases by 0 02 on the average for each centigrade decrease in temperature Accordingly the value of NCE should be about 3 5 mEq/100 g protein lower at 20°C than at 38 C, other conditions remaining equal.

In view of the accuracy needed the experiments of Van Slyke are not easily repeated and it is therefore not surprising to find that only two other attempts have been made to determine the NCE of the plasma proteins.

In 1929 Darrow & Hartmann pointed out that equation (B) of Van Slyke and co-workers could not be used to calculate 'base binding power in patients in whom the A G ratio was abnormal They did therefore some experiments with beef serum which had been dialysed for 2 to 3 days against distilled water in an atmosphere of CO_2 and in the cold During the dialysis part of the globulins precipitated

After dialysis CO_2 titration after the manner of Van Slyke *et al* was carried out separately on whole serum, on the washed precipitated globulins and on the proteins not precipitated by dialysis. Total protein was determined by the macro-Kjeldahl method using factor of 6.25 Albumin and globulin were determined in the same way after fractionation by 22.2 per cent disodium sulfate Apparently two samples were studied, both at two pH values (about 7.2 and about 7.5)

(for the pH range covered by the experiments)

$$BP = 0.068\ P_s\ (pH - 4.80) \qquad (A)$$

where BP represents base bound by protein (*i.e.* NCE) in mEq and P_s serum protein in grams. (The pH at which BP would be zero according to the equation – in this case 4.80 – will be indicated as apparent IEP'). According to equation (A) the NCE of 100 g of normal horse protein is 17.4 mEq at pH 7.35 and increases by 6.8 mEq for every 1.0 unit increase in pH. It is interesting to note that the values used in the general equations from which the authors calculated the Donnan ratios across the erythrocyte membrane and across the capillary wall are based on this equation.

In 1927, in a paper concerned with the distribution of electrolytes between transsudates and serum, HASTINGS *et al.* (1927) pointed out that equation (A) correlated BP with total protein only and that – as the titration curves of albumin and globulin differed greatly and moreover the albumin/globulin ratio was not the same in man as in the horse – the equation could not be used to calculate the base bound by human serum proteins. They mentioned experiments in which the method described above was applied to dialysed human serum. From these experiments the following relation was calculated for human serum

$$BP = 0.097\ P\ (pH - 5.26) \qquad (B)$$

Equation (B) indicates a NCE of 20.3 mEq per 100 g of (normally constituted) human serum at a pH of 7.35, increasing with 9.7 mEq for every 1.0 pH unit increase. The experimental data from which equation (B) was derived have never been published as far as we could ascertain.

Instead, another equation was proposed in 1928 by VAN SLYKE *et al.* It is based on the results obtained with albumin and globulin fractions of horse serum. The globulin was salted out from serum with half saturated $(NH_4)_2SO_4$, albumin with saturated $(NH_4)_2SO_4$, and both fractions were freed from small electrolytes by pressure dialysis against distilled water which had been saturated with CO_2 to bring its reaction near to the isoelectric point of the protein fractions. In this study the protein solutions were not only titrated with CO_2, but with HCl also. For that purpose the protein fractions were dissolved in enough NaOH to render the solution distinctly alkaline and then titrated electrometrically with 0.1 N HCl. The amount of cation opposed by protein valencies was found by subtracting the HCl added from the NaOH originally present in the solution.

In three experiments 2 preparations of albumin (protein concentration about 50 g/Kg H_2O) were studied, and in another three experiments 2 preparations of globulin (protein concentration 70.9 and 36.2 g/Kg H_2O). The Na concentration was between 135 and 139 mEq/Kg H_2O and all experiments were done at 38°C. Determinations were done at 3 to 7 different pH values, ranging from 5.20 to 9.26, but in the final analysis the authors have only included the values obtained at pH values between 6.50 and 7.80. In the calculation of HCO_3 a value of 6.11 was assumed for pK_1 of carbonic acid.

The relation between pH and BP was again a linear one for both protein fractions and no difference was found between the results obtained with CO_2 and with HCl titration.
From their results the following equation was calculated for horse serum

$$BP = 0.124\ Alb\ (pH - 5.16) + 0.077\ Glob\ (pH - 4.89) \qquad (C)$$

In their publication the protein values were expressed in grams of N. To make the equation comparable with the older ones we have expressed it in terms of grams of protein assuming an N factor of 6.25 as this was the factor which Van Slyke used for calculating equation (D).

In normal human serum Van Slyke *et al* found with their fractionation technique the ratio albumin-globulin to be 1 6. From (C) they calculated therefore for normal human serum the equation

$$BP = 0.104\,P\ (pH - 5.08) \quad (D)$$

According to (D) the "base binding power" that is the NCE, of 100 g human serum protein (at pH 7.35 and 38°C) is 23.6 mEq and increases 10.4 mEq per unit pH

The correctness of extrapolating the values found with horse serum proteins to human serum was tested with one sample of pooled human serum which had been dialyzed for three months at 0°C. It contained 17.2 g/Kg H_2O albumin and 14 4 g/Kg H_2O globulin The CO_2 titration was carried out at 4 different values of pH (6.01 to 7.97), the Na concentration was 136 mEq/Kg H_2O and the temperature 38°C. At every pH the BP found was 5–7 % lower than the value calculated from equation (D) At the end of their article Van Slyke *et al* commented on the fact that the earlier equations indicated a considerably lower value for BP They concluded that probably in the earlier experiments dialysis had not been pushed far enough to remove the last traces of pre-existing base from the protein preparations used

Equation (C) and (D) have generally been accepted, and the value of 16 mEq/L inserted in the usual cation-anion diagrams for plasma or serum is based on them. Table 1 summarises the results obtained in the experiments of Van Slyke and co-workers. The NCE values were calculated for pH 7.35 and expressed in mEq per 100 g *protein*. This was *done* because in our experiments to be discussed later all values have been expressed per 100 g protein. The pH 7.35 was selected because generally not arterial but venous blood is analyzed, which in the resting forearm appears to have a pH of on the average 7 35

It should be stressed that Van Slyke *et al.* did their experiments at 38°C. The *observations* of S[illegible] *et al* (1925) on the effect of temperature on acid-base-protein equilibrium in the pH range 7 00–7 70 suggest that whereas the molecular buffer value of the plasma proteins is not affected when the temperature changes from 38°C to 20°C, the apparent IEP increases by 0 02 on the average for each centigrade decrease in temperature. Accordingly the value of NCE should be about 3 5 mEq/100 g protein lower at 20°C than at 38°C, other conditions remaining equal.

In view of the accuracy needed the experiments of Van Slyke are not easily repeated and it is therefore not surprising to find that only two other attempts have been made to determine the NCE of the plasma proteins.

In 1929 Darrow & Hartmann pointed out that equation (B) of Van Slyke and co-workers could not be used to calculate "base binding power" in patients in whom the A G ratio was abnormal. They did therefore some experiments with beef serum which had been dialysed for 2 to 3 days against distilled water in an atmosphere of CO_2 and in the cold During the dialysis part of the globulins precipitated.

After dialysis CO_2 titration after the manner of Van Slyke *et al.* was carried out separately on whole serum, on the washed precipitated globulins and on the proteins not precipitated by dialysis. Total protein was determined by the macro-Kjeldahl method using a factor of 6 25 Albumin and globulin were determined in the same way after fractionation by 22.2 per cent disodium sulfate Apparently two samples were studied, both at two pH values (about 7.2 and about 7 5)

The globulins precipitated during dialysis were assumed to be representative for all globulins and from the CO_2 titration of the precipitated fraction the following equation was obtained for bovine globulin

$$B\ Glob = 0.04\ Glob\ (pH - 5.58)$$

where B Glob stands for base binding power of the globulins (in mEq) and Glob for grams of globulin. With the aid of the above equation and knowing the concentration of albumin and globulin as well as the base binding power of (dialysed) whole serum the following equation was obtained for bovine albumin

$$B\ Alb = 0.141\ Alb\ (pH - 5.42)$$

The equations given by Darrow and Hartmann were rearranged by us in the notation used by Van Slyke and co workers The NCE values calculated by means of these equations are presented in table 1

No details concerning the experiments are given but it is mentioned that after dialysis the serum still contained some calcium. The authors also pointed out that the equations are probably not correct because of the assumption of equal value of base binding power for precipitated and non precipitated globulin, and because of the subtractions necessary to obtain the equations for albumin No data are given for the normal A/G ratio found with the fractionation technique used But if we assume the ratio to be the same as that found by VAN SLYKE *et al* in 1928 (*i e* 1 6) we calculate from the equations given by Darrow and Hartmann a value of 19.5 mEq/100 g protein for the NCE of normal bovine serum protein This is very nearly the value obtained for human serum from equation (B) of Van Slyke and coworkers

HENDERSON *et al* (1931) have applied the same method (*i.e* CO titration) to 10 samples of human plasma obtained from 6 normal persons. But their results are sparsely documented and lend themselves only to the calculation of the buffer value as the authors have assumed an arbitrary value for NCE at pH 7 40 They calculate for the pH range 7 0 to 7 8 a mean buffer value of 10 9 (expressed in mEq increase per 100 g protein per unit pH) (table 1)

It is at times difficult to understand what precisely the authors have been doing, as practically no experimental data are given and only a composite graph and table are reproduced. Furthermore no mention is made of how the plasma was obtained and in what manner it was further treated. It is not even clear whether the samples were subjected to dialysis prior to the titration experiments.

The results of the titration curves obtained for purified albumin (chapter I) can also supply information on the buffer value only Recent data on bovine serum albumin (TANFORD *et al* 1955) suggest that at an ionic strength of 0 15 the buffer value in the pH range 7 0 to 8 0 is about 12.0 when expressed in mEq per 100 g albumin per unit pH This value we derived from the titration curve published by Tanford and it is therefore admittedly an estimate, but it agrees rather well with the buffer value found by Van Slyke and co workers for horse albumin which was 12.4 (equation (C)) In a similar manner we calculated from KEPES titration curves for bovine serum albumin a buffer value of approximately 11 0 in the pH range 7 0 to 7 5 (titration in the presence of 29 mEq/L KCl) For the globulins no such titration data exist.

The above mentioned data suggest that in so far as the buffer value is concerned

equations (C) and (D) of Van Slyke and co-workers are probably near the truth. For the absolute value of NCE no determinations are available more recent than those published by Van Slyke *et al* in 1928

Objections to the application of the results obtained by Van Slyke et al. to human plasma
While reading the publications of Van Slyke and associates, especially the one from 1928, one is impressed by the detailed presentation of the experimental data and the obviously high accuracy of the analytical techniques. But there are several objections to generalising the results of these experiments

(*a*) The number of protein preparations studied was very small indeed and nothing is therefore known about the physiological variation.

(*b*) The final equation was derived from experiments on horse protein, and in the one instance in which it was tested with human serum preparations the latter had been dialysed for 3 months at 0°C.

(*c*) According to present knowledge the technique used for fractionating the proteins is a very rough one, both in the sense that the fractions obtained are not comparable to electrophoretically homogeneous albumin and globulin, and in the sense that the treatment might easily have damaged the protein molecules.

(*d*) The titrations were done in a solution of physiological ionic strength, but containing univalent cations only Since the interaction of proteins with bivalent ions is quite different, it remains to be proven whether the results obtained with a solution containing Na as only cation are applicable to plasma without restriction. From the observations of Kepes discussed in chapter I one must conclude that this is not so

(*e*) On the assumption that Na was the only cation present in the solution, Van Slyke *et al* could equate the known amounts of Cl and HCO_3 against a known total amount of cations and therefore calculate the amount of cations opposing the only other anion present, *i e* protein. However as discussed in chapter I, protein also figures on the cationic side of the ion balance In reality therefore the cation-anion balance was $[Na^+] + [Pr^+] = [Pr^-] + [Cl^-] + [HCO_3^-]$ and since this equation contains two unknown quantities the cation and the anion equivalency – it can not be solved. This stresses again the fact that the values found by Van Slyke *et al* refer to the *net* cation equivalency

(*f*) Finally although only NCE was determined, the values given by Van Slyke are tacitly accepted as indicating net negative charge. The difference has been pointed out in chapter I

ESTIMATION OF NCE FROM THE VALUES REPORTED FOR THE NORMAL PLASMA CONCENTRATIONS OF CATIONS AND ANIONS OTHER THAN PROTEIN

Theoretically for any plasma sample NCE of plasma protein can be derived from the difference between the sum concentration of all cations other than protein and that of all anion other than protein. In practice no accurate results are obtained for several reasons Firstly the precise contribution of organic acids to the ionic balance is not known. But as it is small compared with the NCE of the proteins, this need not be a serious objection. A reasonable estimate of its magnitude should be possible from the normal values published for lactate citrate ketonbodies, non-esterified fatty acids and

amino acids The fact that on the cation-side the ammonium ions are neglected is without influence since even in pathological conditions *their total amount is never more than* 0 1 mEq/L. The second difficulty encountered is more serious and is due to the errors in chemical determination *A positive error of 1 / in the* Na determination and a negative error of 1 / in Cl analysis cumulate in overestimating NCE by something like 15 /. *The high accuracy achieved by Van Slyke et al* in their experiments is partly due to the fact that the concentrations of Na and Cl were accurately known, as the solutions on *which the determinations* were done were prepared by the authors

It should nevertheless be possible to estimate with the aid of the normal values published for cations and anions in plasma whether Van Slyke *et al* s most recent value for NCE is of the right order of magnitude With this end in mind table 2 was compiled

Presentation of data in table 2

In principle we have only listed data which are documented reasonably well, but a few undocumented ones have also been included either because of the authority of the authors (GAMBLE, PETERS) or because they belong to the small number of investigations in which the cations as well as the anions were determined on the same plasma samples (JEANNERET *et al* 1954) Because they are always quoted the values given by Gamble in his classic lecture syllabus on the composition of the extracellular fluid head the list although we have been unable to find the experimental data on which they rest

For historical reasons it must be mentioned that SCHMIDT (1850), apparently the first to determine Na and Cl in human serum, found in the two normal sera analysed a Na concentration of 148.0 respectively 138.0 mEq/L and a Cl concentration of 100 0 respectively 102.4 mEq/L. For K values of about 8 0 mEq/L were found, presumably as a result of damage to the erythrocytes during preparation of the serum.

The weight of the values listed in table 2 is roughly indicated by the number of asterisks in the last column of the table. No asterisk signifies that the values were reported without, or with scant documentation one asterisk *indicates a reasonable degree of documentation* two asterisks detailed documentation In this respect special attention has been given to Na and Cl For Na (and for total base) a variety of analytical methods have been used. These are indicated in table 2. Cl was nearly always determined by one of the modifications of the open Carius method, the exception being *the study by* FAWCETT & WYNN who made use of potentiometric titration HCO_3 has always been calculated from total CO_2 and pH (where the latter was generally assumed to be 7 35)

Concerning the composition of table 2 the following must be said On the side of the cations we have listed separately the concentration of the individual cations, *the sum of* these concentrations and the concentration of total base. Total base has been determined more frequently than the sum concentration of the individual cations *Moreover in* calculating the latter some authors had to assume a value for the concentration of Mg. In that case the value listed for the sum concentration of the cations *has been placed* between brackets

On the side of the anions the concentrations *of Cl of HCO_3 and of inorganic phos*phate are listed with the sum concentration of Cl + HCO_3 SO_4 and organic acids were not determined in the studies mentioned but an estimate *of the normal concentration* of these substances can be made from the literature (table 3)

Rest anions. It would appear that the average normal value for plasma SO_4 concentration is 0.7 mEq/L, and that normally the organic acids contribute about 3.0 mEq/L to the anionic valencies in plasma and nothing to the cationic valencies. The value of 3.0 is based on the assumption that the sum of the equivalent concentrations of all anionic 'trace' substances is about 1.0 mEq/L. The amino acids were listed with these 'trace' anions because at normal plasma pH their positive and negative valencies will nearly balance each other (the normal plasma concentration of filtrable amino acids is only about 2.5 mM/L (WALKER *et al.* 1962)).

As the normal plasma concentration of inorganic phosphate was on the average 2.2 mEq/L in the studies listed in table 2, we have estimated that inorganic phosphate, sulfate and organic acid together (the socalled *rest anions* or *RA*) contribute on the average 6.0 mEq/L to the anionic valencies in normal plasma.

We have therefore subtracted from the values listed under the heading (cat minus Cl + HCO_3) in table 2 the value of 6.0 in order to obtain an estimate of NCE. The resulting values have been listed in the corresponding column.

In the cation-anion diagram of normal plasma used by Gamble in his famous lectures on extracellular fluid, the value of NCE is given as 16 mEq/L, *i.e.* the value calculated with the aid of equation (D) of Van Slyke and co-workers. The concentration of organic acids is given by Gamble as 6.0 mEq/L since this amount made the total anionic valency in Gamble's diagram equal to the total cationic valency. However if we consider NCE as the unknown quantity and assume the contribution of the organic acids to be 3.0 mEq/L (see before), we calculate from the concentrations which Gamble reports for the other ions, NCE value of 19.0 mEq/L. This is the value listed in table 2.

Discussion of the data presented in table 2

The first fact which becomes apparent on studying the contents of table 2 is the small number of studies in which a sufficiently large number of the various cations and anions were determined to allow an estimate of NCE to be made. Furthermore the three studies in which Na and K were determined by flame photometry are either sparsely documented (DANOWSKI *et al.* 1955, ANNINO & RELMAN 1959) or not at all (JEANNERET *et al.* 1954).

The second fact is the large variation in the values reported for the normal plasma or serum Na concentration even after the introduction of the flame photometer. The variation in the reported values for total base is smaller but the values still cover a range of about 10 mEq/L. The values reported for Cl on the other hand have been fairly constant throughout the years. Clearly a reasonable estimate of NCE can only be made from those studies in which cations (or total base) and anions were both determined in the same plasma or serum samples. In ten of the reported studies this appears to have been the case. Seven of these were published before 1940. But by assuming for Mg a normal plasma value of 2.0 mEq/L, the results of JEANNERET *et al.* (1954), of DANOWSKI *et al.* (1955) and of ANNINO & RELMAN (1959) could also be used for the calculation of NCE. (From the series of Danowski *et al.* only the values for adults were taken. The Cl and HCO_3 values for males and for females have been averaged).

If we consider the NCE values obtained from these selected series, we find that they vary from 10 to 25 mEq/L plasma whereas the protein concentration only varies from 65.0 to 74.8 g/L. The large spread in NCE is apparently a result of the large spread in the values found for the sum concentrations of the cations. Furthermore it can be seen that in the studies published since 1930 the value estimated for NCE is in general below the value expected from equation (D) of Van Slyke and co-workers. Several authors have commented on the discrepancy

amino acids. The fact that on the cation-side the ammonium ions are neglected is without influence, since even in pathological conditions their total amount is never more than 0 1 mEq/L. The second difficulty encountered is more serious and is due to the errors in chemical determination. A positive error of 1 / in the Na determination and a negative error of 1 / in Cl analysis cumulate in overestimating NCE by something like 15 /. The high accuracy achieved by Van Slyke *et al* in their experiments is partly due to the fact that the concentrations of Na and Cl were accurately known, as the solutions on which the determinations were done, were prepared by the authors

It should nevertheless be possible to estimate with the aid of the normal values published for cations and anions in plasma whether Van Slyke *et al* s most recent value for NCE is of the right order of magnitude With this end in mind table 2 was compiled

Presentation of data in table 2

In principle we have only listed data which are documented reasonably well, but a few undocumented ones have also been included either because of the authority of the authors (GAMBLE, PETERS) or because they belong to the small number of investigations in which the cations as well as the anions were determined on the same plasma samples (JEANNERET *et al* 1954) Because they are always quoted the values given by Gamble in his classic lecture syllabus on the composition of the extracellular fluid head the list, although we have been unable to find the experimental data on which they rest.

For historical reasons it must be mentioned that SCHMIDT (1850) apparently the first to determine Na and Cl in human serum, found in the two normal sera analysed a Na concentration of 148 0 respectively 138 0 mEq/L and a Cl concentration of 100.0 respectively 102.4 mEq/L. For K values of about 8 0 mEq/L were found, presumably as a result of damage to the erythrocytes during preparation of the serum.

The weight of the values listed in table 2 is roughly indicated by the number of asterisks in the last column of the table No asterisk signifies that the values were reported without or with scant, documentation one asterisk indicates a reasonable degree of documentation two asterisks detailed documentation In this respect special attention has been given to Na and Cl For Na (and for total base) a variety of analytical methods have been used These are indicated in table 2. Cl was nearly always determined by one of the modifications of the open Carius method the exception being the study by FAWCETT & WYNN who made use of potentiometric titration HCO_3 has always been calculated from total CO_2 and pH (where the latter was generally assumed to be 7.35)

Concerning the composition of table 2 the following must be said On the side of the cations we have listed separately the concentration of the individual cations, the sum of these concentrations and the concentration of total base. Total base has been determined more frequently than the sum concentration of the individual cations. Moreover in calculating the latter some authors had to assume a value for the concentration of Mg. In that case the value listed for the sum concentration of the cations has been placed between brackets.

On the side of the anions the concentrations of Cl of HCO_3 and of inorganic phosphate are listed with the sum concentration of Cl + HCO_3 SO_4 and organic acids were not determined in the studies mentioned but an estimate of the normal concentration of these substances can be made from the literature (table 3)

They determined Na (by modification of the uranyl zinc acetate method), K, Ca, HCO_3, Cl, PO_4, total protein and protein fractions and calculated the NCE with the aid of equation (C) of Van Slyke *et al.* Mg was assumed to be 2 mEq/L. For every serum sample they calculated the difference between the sum concentration of the cations on the one hand and the sum concentration of Cl, HCO_3, phosphate and (calculated) NCE on the other and the values thus obtained were plotted against the euglobulin content of the serum.

The results are shown in fig. 8. It can be seen that the cation-anion difference – which ought to represent SO_4 + organic acids – ranged from 4.0 to — 0.5 mEq/L for the 10 normal persons investigated, leaving the somewhat low average value of about 2 mEq for the sum concentration of SO_4 and organic acids. More impressive however is the

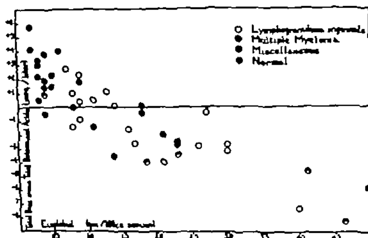


Fig. 8. Correlation between (total base minus total determined acids) and euglobulin content of serum. See text for explanation. Reproduction of fig. 1 in publication of Olitsky *et al.* (1936) J. clin. Invest. *15* 475

evident negative correlation which existed between the amount of euglobulin per 100 cc serum and "total base minus total determined acids" the latter assuming rather large negative values at high concentrations of euglobulin. The authors concluded that obviously equation (C) of Van Slyke *et al.* cannot be used when the protein composition is quantitatively and qualitatively pathological. In the diseases studied the euglobulin fraction evidently had a far lower NCE than the globulin fraction studied by Van Slyke *et al.*

Possible causes for the observed variation in the estimated values of NCE

(a) *The large variation in the values reported for normal plasma Na concentration and total base*

From table 2 it will be seen that the Na values obtained with the pyroantimonate method are rather high, with the notable exception of those reported by Marrack. The values found with the gravimetric uranyl zinc acetate method are somewhat lower and vary from 135.0 to 142.5 some authors reporting consistently low values. The controversy between Hald and Consolazio *et al.* has already been mentioned. Contrary to what

ATCHLEY & BENEDICT (1930) determined total base, chloride, bicarbonate and phosphate in the serum of 10 normal adults and of a number of patients They found the value for total base rather low in comparison to the values published earlier Inserting for NCE the value calculated with the aid of equation (D) into the cation-anion diagram for serum they observed that in the normal persons studied on the average only 0.1 mEq/L plasma was left for SO_4 and all organic anions together No conclusion was drawn as to the validity of equation (D) but in the introduction to their experimental results they stated that the equations (*i e* for calculating base bound by the proteins) are based on data so difficult to obtain that they undoubtedly will be altered by further study

PETERS called attention to the fact that in certain patients with the nephrotic syndrome the concentration of Cl in the serum was abnormally high and the estimated sum of anionic equivalents (which included NCE calculated with the aid of equation (D)) exceeded the observed concentration of total base He postulated that part of the serum Cl was loosely bound by the serum lipids He has tried to determine this lipoid-chlorine by extracting the serum lipids with hot alcohol and ether (PETERS & MAN 1934) In normal persons the extracts contained 2.7 to 5 7 mEq/L Cl and in patients with increased serum lipids this amount could be as high as 11 mEq But the correlation between lipoid-chlorine concentration and lipid content of the plasma was a very rough one, as was the correlation between the concentration of lipoid-chlorine and apparent base deficit. Peters pointed out that the methodical difficulties were such that no quantitative conclusions were possible and he promised further experiments on this interesting phenomenon However no further evidence has emerged and to our knowledge lipoid chlorine has not been mentioned in later publications

The conclusion of Peters that in a number of persons the cation-anion diagram of plasma indicates apparently a cation deficit or anion excess (*i.e.* when for NCE the value calculated with the aid of equation (C) or equation (D) was inserted) was based on the analyses done by HALD She developed a modification of the Stadie and Ross method for the total base determination, and a rather intricate fractionation procedure to obtain Na from ashed serum, the Na being determined gravimetrically after precipitation with uranyl zinc acetate. Her normal values (1933) for total base and Na were considerably lower than those published before that time, with the exception of those found by SUNDERMAN (1930). The validity of her results has been questioned by CONSOLAZIO & TALBOTT (1940) They found distinctly higher values for total base (with electrodialysis and with the iodometric total base determination of VAN SLYKE *et al* (1927)), and for Na (with a modification of the uranyl zinc acetate method). But on the same samples, the method of HALD gave in their hands practically the same low values as Hald had found herself They concluded that with Hald's technique Na was co-precipitated during the preliminary precipitation of phosphate and they virtually proved their contention in 1941 (CONSOLAZIO & DILL 1941). Hald could not confirm this and concluded from experiments with ²⁴Na that no Na got lost during phosphate precipitation (1946). However from her experimental data it is evident that some radioactivity was found in the precipitated phosphate and in the same publication she reported normal Na values, which are about 2 mEq/L higher than those she published in 1933 and in 1937 (HALD & EISENMANN 1937). Even so they remain low in comparison to most other values published including the ones later obtained with flame photometry

That in a number of patients the cation-anion diagram apparently indicates an anion excess, if equation (C) or (D) of Van Slyke *et al* are used for calculating NCE, was clearly demonstrated by GUTMAN *et al* (1936) They studied the acid base equivalency of the serum in patients with hyperproteinaemia due to a very high euglobulin content of the serum (Howe's method was used for the protein fractionation)

The 'protein interference effect' has also been observed for K (BERNSTEIN 1952, BERGSTRÖM & HULTMAN 1962, BERGMAN *et al.* 1963).

BERNSTEIN has apparently been the first to observe difference between the flame photometer values obtained with plasma and with deproteinised plasma. He attributed this to an increase in the volume occupied by protein as result of trichloroacetic acid binding by the protein.

The above cited observations apparently point to the value obtained for deproteinised plasma as representing the true Na concentration. But it must be doubted whether this conclusion is correct.

SACKNER & SUNDERMAN (1958) report very low Na values although they analysed deproteinised plasma (Beckman flame photometer). On the other hand among the highest normal Na values reported are those found by BERNSTEIN (1952) and DANOWSKI *et al.* (1955) who analysed plasma. The validity of the results obtained with neutron activation analysis is questionable (VAN LEEUWEN *et al.* 1963). I the observation reported by BERGMAN *et al.* (1963) the spread found for ^{24}Na is nearly thrice as large as that found for ^{22}Na. Moreover if the values obtained for ^{24}Na are correct the average plasma Na concentration of the 14 normal subjects studied by Bergman *et al.* would hav been rather high, *i.e.* 145.4 mEq/L. Furthermore Bergstrom and Hultman appear not to have corrected the values obtained for deproteinised plasma for the effect of protein removal on the concentration of the remaining serum constituents.

Clearly further experiments are necessary to decide whether analysis of deproteinised plasma (or serum) gives more accurate results than the analysis of plasma (or serum) directly.

From this survey it appears that there is no common operator which can explain the differences between the values reported for normal plasma Na concentration. It is therefore unjustifiable to average the various values, and we must conclude that the accurate value of the Na concentration in normal plasma or serum is still a matter of conjecture.

The values reported for total base lead to a somewhat similar conclusion. But there the variation is less and the only two authors who have reported remarkably low values re HALD and SUNDERMAN. With SACKNER the latter has published in 1958 the results of a study in which the Na concentration has been determined both with the gravimetric uranyl zinc acetate method and with the flame photometer and the total base concentration by 4 methods including Sunderman's procedure for measuring conductivity (1945) and Hald's method. Only 5 normal sera were investigated but in these and in the sera of 21 patients with a variety of diseases, low values for the concentration of both Na and total base were found with all methods used. However the agreement between the conductivity procedure and Hald's method is not wholly unexpected, since the first appears to ha e been standardised against the latter (SUNDERMAN 1942). Since Sunderman results are not so well documented as those of Hald or Consolazio an explanation of the d fference with the values reported by others is not feasible.

(b) *Variations in condition of subject at moment of sample taking*

The results obtained by the various authors may have been influenced by the condition of the persons whose blood was studied. WALAAS & WALAAS (1949) found total base to increase some 5 mEq/L as a result of breaking fast by a (normal?) meal. On the other hand ANNINO & RELMAN (1959) found only a slight increase in serum Na concentration after breakfast, whereas FAWCETT & WYNN (1956) saw no change after a meal.

might be expected the Na values obtained with the flame photometer show a larger instead of a smaller spread than the values obtained by chemical methods This is partly due tot the fact that after the introduction of the flame photometer the determination of Na and K seemingly became a *simple matter* *Thus has sometimes led to a somewhat* uncritical acceptance of results which are definitely inferior to those obtained by previous workers with the more laborious techniques. ELLIOT & HOLLEY (1951) determined serum Na in a 1 50 dilution with a Perkin Elmer flame photometer using the method of MOSHER *et al* (1949) The latter added gelatin to their standard solutions, but not to the *plasma and from their data it is evident that* gelatin in the concentration used has a depressive effect on the flame photometer values obtained both for Na and for K. The standard solutions give therefore too low a galvanometer reading and as a consequence the plasma values are overestimated. The exceedingly high value reported by OVERMAN & DAVIS (1947) was obtained on protein free filtrate of plasma in a 1 60 dilution and *also* with a *Perkin Elmer* The anticoagulant used is not mentioned and since some heparin preparations contain a very high concentration of Na this might have caused a positive error The rather high value reported by DANOWSKI *et al* (1955) was obtained on serum using a Patwin flame photometer and a serum dilution of 1 250 (personal communication of DANOWSKI 1962) the intermediate values reported by JEANNERET *et al* (1954) are not documented. The value reported by TARAIL *et al* (1952) was obtained with a Barclay flame photometer using an internal standard and dilutions of 1 200 and 1 400 MOHUN & COOK (1962[1]) found the very low value of 135 0 for normal plasma samples in 1 500 dilution with the EEL flame photometer The heparin used by them contained no Na. The very high dilution seems to preclude any disturbing influence due to viscosity and yet their values are very much lower than those of Roegholt, who used a dilution of 1 25 In ROEGHOLT s study (1955) Na-containing heparin was used as *anti-coagulant, but this contributed only 0 5 mEq/L to the* reported Na value.

Of special interest are the observations of BERGSTRÖM & HULTMAN (1962) who determined Na concentration with an Eppendorf flame photometer both in plasma and in deproteinised plasma (protein precipitated with trichloroacetic acid) Plasma Na concentration was also determined by means of neutron activation analysis (BERGSTRÖM 1962). *Flame photometer analysis of deproteinised plasma resulted* in Na concentrations which were on the average 6 5 mEq/L higher than those found when analysing plasma. As the values found with neutron activation were in good agreement *with those obtained* for deproteinised plasma the authors concluded that the latter are correct. They suggested that the very low values reported by Mohun and Cook were due to a protein interference effect *The latter presumably varies* according to the flame photometer used. In the ensuing discussion MOHUN & COOK (1962[2]) reported that they had subsequently found a protein interference effect of the same order as Bergström and Hultman. In their original experiments they had been misled by the fact that commercial control sera agreed precisely with their own standards, but had now observed that contrary to fresh *serum*, these control sera showed no protein interference effect BERGMAN *et al* (1963) reported experiments in which ^{24}Na was added to heparinised plasma and the latter then subjected to ultrafiltration Plasma and ultrafiltrate were analysed both for ^{24}Na (with a G-M tube) and for ^{23}Na *(with an Eppendorf flame photometer) and* it was found that the ratio ^{24}Na/^{23}Na was higher in plasma than in the ultrafiltrate The authors concluded that this observation was further evidence for the protein interference effect

The 'protein interference effect has also been observed for K (BERNSTEIN 1952, BERGSTRÖM & HULTMAN 1962, BERGMAN *et al* 1963).

> BERNSTEIN has apparently been the first to observe a difference between the flame photometer values obtained with plasma and with deproteinised plasma. He attributed this to an increase in the volume occupied by protein as a esult of trichloroacetic acid binding by the proteins.

The above cited observations apparently point to the value obtained for deproteinised plasma as representing the true Na concentration. But it must be doubted whether this conclusion is correct.

> SACKNER & SUNDERMAN (1958) report very low Na values although they analysed deproteinised plasma (Beckman flame photometer). On the other hand among the highest normal Na values reported are those found by BERNSTEIN (1952) and DANOWSKI *et al.* (1955) who analysed plasma. The validity of the results obtained with neutron activation analysis is questionable (VAN LEEUWEN *et al* 1963). In the observation reported by BERGMAN *et al.* (1963) the spread found for ^{24}Na is nearly thrice as large as that found for ^{23}Na. Moreover if the values obtained for ^{24}Na are correct the verage plasma N concentration of the 14 normal subjects studied by Bergman *et al.* would be been rather high, 145 4 mEq/L. Furthermore Bergstrom and Hultman appear not to have corrected the values obtained for deproteinised plasma for the effect of protein removal on the concentration of the remaining serum constituents.

Clearly further experiments are necessary to decide whether analysis of deproteinised plasma (or serum) gives more accurate results than the analysis of plasma (or serum) directly

From this survey it appears that there is no common operator which can explain the differences between the values reported for normal plasma Na concentration. It is therefore unjustifiable to average the various values, and we must conclude that the accurate value of the Na concentration in normal plasma or serum is *still* a matter of conjecture.

The values reported for total base lead to a somewhat similar conclusion. But there the variation is less and the only two authors who have reported remarkably low values are HALD and SUNDERMAN With SACKNER the latter has published in 1958 the results of a study in which the Na concentration has been determined both with the gravimetric uranyl zinc acetate method and with the flame photometer and the total base concentration by 4 methods including Sunderman's procedure for measuring conductivity (1945) and Hald's method. Only 5 normal sera were investigated but in these and in the sera of 21 patients with a variety of diseases, low values for the concentration of both Na and total base were found with all methods used. However the agreement between the conductivity procedure and Hald's method is not wholly unexpected, since the first appears to have been standardised against the latter (SUNDERMAN 194) Since Sunderman's results are not so well documented as those of Hald or Consolazio an explanation of the difference with the values reported by others is not feasible.

(b) *Variations in condition of subject at moment f sample taking*

The results obtained by the various authors may have been influenced by the condition of the persons whose blood was studied. WALAAS & WALAAS (1949) found total base to increase some 5 mEq/L as a result of breaking fast by a (normal?) meal. On the other hand ANNINO & RELMAN (1959) found only a slight increase in serum Na concentration after breakfast, whereas FAWCETT & WYNN (1956) saw no change after a meal

might be expected the Na values obtained with the flame photometer show a larger instead of a smaller spread than the values obtained by chemical methods. This is partly due tot the fact that after the introduction of the flame photometer the determination of Na and K seemingly became a simple matter. This has sometimes led to a somewhat uncritical acceptance of results which are definitely inferior to those obtained by previous workers with the more laborious techniques. ELLIOT & HOLLEY (1951) determined serum Na in a 1 : 50 dilution with a Perkin Elmer flame photometer using the method of MOSHER et al. (1949). The latter added gelatin to their standard solutions but not to the plasma and from their data it is evident that gelatin in the concentration used has a depressive effect on the flame photometer values obtained both for Na and for K. The standard solutions give therefore too low a galvanometer reading and as a consequence the plasma values are overestimated. The exceedingly high value reported by OVERMAN & DAVIS (1947) was obtained on protein free filtrate of plasma in a 1 : 60 dilution and also with a Perkin Elmer. The anticoagulant used is not mentioned and since some heparin preparations contain a very high concentration of Na this might have caused a positive error. The rather high value reported by DANOWSKI et al. (1955) was obtained on serum using a Patwin flame photometer and a serum dilution of 1 : 250 (personal communication of DANOWSKI 1962); the intermediate values reported by JEANNERET et al. (1954) are not documented. The value reported by TARAIL et al. (1952) was obtained with a Barclay flame photometer using an internal standard and dilutions of 1 : 200 and 1 : 400. MOHUN & COOK (1962[1]) found the very low value of 135.0 for normal plasma samples in 1 : 500 dilution with the EEL flame photometer. The heparin used by them contained no Na. The very high dilution seems to preclude any disturbing influence due to viscosity and yet their values are very much lower than those of Roegholt, who used a dilution of 1 : 25. In ROEGHOLT's study (1955) Na-containing heparin was used as anti-coagulant, but this contributed only 0.5 mEq/L to the reported Na value.

Of special interest are the observations of BERGSTRÖM & HULTMAN (1962) who determined Na concentration with an Eppendorf flame photometer both in plasma and in deproteinised plasma (protein precipitated with trichloroacetic acid). Plasma Na concentration was also determined by means of neutron activation analysis (BERGSTRÖM 1962). Flame photometer analysis of deproteinised plasma resulted in Na concentrations which were on the average 6.5 mEq/L higher than those found when analysing plasma. As the values found with neutron activation were in good agreement with those obtained for deproteinised plasma the authors concluded that the latter are correct. They suggested that the very low values reported by Mohun and Cook were due to a "protein interference effect". The latter presumably varies according to the flame photometer used. In the ensuing discussion MOHUN & COOK (1962[2]) reported that they had subsequently found a "protein interference effect" of the same order as Bergström and Hultman. In their original experiments they had been misled by the fact that commercial control sera agreed precisely with their own standards, but had now observed that, contrary to fresh serum, these control sera showed no protein interference effect. BERGMAN et al. (1963) reported experiments in which ^{24}Na was added to heparinised plasma and the latter then subjected to ultrafiltration. Plasma and ultrafiltrate were analysed both for ^{24}Na (with a G-M tube) and for ^{23}Na (with an Eppendorf flame photometer) and it was found that the ratio ^{24}Na/^{23}Na was higher in plasma than in the ultrafiltrate. The authors concluded that this observation was further evidence for the "protein interference effect".

lend themselves to a more accurate interpretation. The one study in which a definite attempt was made to check the validity of equation (C) of Van Slyke *et al* in cases with pathological hyperproteinaemia (GUTMAN *et al* 1936) has already been mentioned. It clearly indicated that pathological globulins may have a very low NCE.

In 1945 BROCH published a study on the regulation of serum electrolytes in which he presented observations on total base (determined by electrodialysis), Cl, HCO_3 and serum protein concentration in a total of 99 patients suffering from a variety of diseases (among which all sorts of febrile and infectious diseases, liver disease, and cardiac insufficiency). In about half the number of patients serum was analysed more than once in the course of the illness. The most remarkable finding was that in a number of patients large and often sudden changes in total base were seen resulting in much lower values than those found in health. Since the values for Cl and HCO_3 remained fairly constant, Broch assumed that large and sudden decreases in NCE were responsible. Apparently its value could become zero or even negative (when a value of 9 mEq/L was accepted for the concentration of the rest anions). Broch explained this peculiar phenomenon by assuming sudden changes in IEP of the plasma proteins in the direction of normal blood pH. In one patient with liver cirrhosis the difference between total base on the one hand and the sum concentration of Cl, HCO_3, and inorganic phosphate on the other was found to be 11.7 mEq. But since the albumin concentration was 25.8 g/L, and the globulin concentration 96.6 g/L (method of Howe), Broch calculated from formula (C) of Van Slyke an expected value for NCE of 25.4. This patient obviously belongs to the group of patients studied by Gutman *et al*. But in practically all the other patients observed by Broch no such protein abnormalities were present. The fact that in these patients NCE seemed to alter considerably within a few days has led the author to the conclusion that this is the expression of a regulatory mechanism which counteracts changes in plasma Na concentrations by changing the degree of dissociation of the proteins. No suggestion is made as to the manner in which these abrupt changes in dissociation are brought about (BROCH 1953).

The presentation and discussion of the technique of electrodialysis with which the values for total base concentration are obtained is rather sketchy and it is to be deplored that apparently no attempt has been made to verify at least some of the very low values found for total base by means of summation of the concentrations of the individual cations.

But apart from the sudden changes observed, the values given by Broch indicate that on the whole the NCE of the plasma proteins was lower in the patients than in his normal persons.

In 1947 HALD *et al* published a study in which they considered the possibility to estimate the concentration of serum Na from the sum concentration of Cl and HCO_3. The material used for this purpose was gathered over a period of 15 years and included both Na values obtained with the method of Hald and with the flame photometer. In normal persons they found the difference between Na concentration and the sum concentration of Cl and HCO_3 to be on the average +5.0 mEq/L, but in all patients studied (42, among whom patients with adrenal insufficiency, diabetic acidosis and severe uraemia) the difference was larger. This contrasts with the findings of Broch, but different types of disease were studied in the two groups.

Summarising it can be concluded that apart from the well documented observations of

But the authors last mentioned did observe that about half an hour after the meal the concentration of Cl usually fell about 2 mEq/L and total CO_2 content increased by a similar amount.

Apparently sex has no influence on the value found for normal plasma Na concentration or total base (DANOWSKI 1955 ROTOHOLT 1955, FAWCETT & WYNN 1956) and the difference originally observed by SALVESEN (1928) has therefore not been corroborated. However the plasma concentration of Cl appears to be somewhat higher in the female than in the male and bicarbonate concentration somewhat lower (HALD *et al* 1947 DANOWSKI *et al* 1955 FAWCETT & WYNN 1956)

As to age the investigations by DANOWSKI *et al* (1955) suggest that the normal value for plasma Na concentration changes somewhat in the course of life. But in most of the studies represented in table 2 the subjects were in the age group 20–50 year and differences in age cannot therefore explain the observed spread in the values reported for normal plasma or serum Na

CONCLUSION

The publication of VAN SLYKE *et al* in 1928 remains the latest source of information on the absolute value of NCE of the plasma proteins An attempt to verify their validity for human plasma with the aid of published data on the difference between the concentration of small cations and of small anions in serum or plasma meets with the difficulty that the normal values reported for Na (and for total base) vary considerably There are some indications that possibly the NCE of the plasma proteins is smaller than the value calculated with the aid of the last reported equation of Van Slyke *et al* but for the moment it cannot be decided whether this is due to an error in the determination of the cation anion balance, or to the fact that the data of Van Slyke are not applicable to human plasma.

NCE OF PLASMA PROTEINS (PATHOLOGICAL PLASMA)

Because of the tendency of some plasma proteins to associate with ions and with a number of molecular substances, a large increase in the plasma concentration of the latter (as seen in uraemia or hyperlipaemia) may well result in a change in NCE of the plasma proteins The effect of adding longchain fatty acids to serum on the electrophoretic characteristics of albumin and lipoproteins (GORDON 1955 also GOODMAN 1958) is rather suggestive in this respect. But because of the analytical difficulties involved very little is known about the NCE in disease beyond the fact that a change in the albumin-globulin ratio does affect its magnitude.

A number of authors have studied the cation-anion balance in plasma in such condition as pneumonia, diabetic acidosis uraemia, nephrosis, etc. (MARRACK 1923 ATCHLEY *et al* 1923 1930 SUNDERMAN *et al* 1926 SUNDERMAN 1930, BLACKFAN & HAMILTON 1927 PETERS *et al.*, 1929[1] 1929[2] SALVESEN 1928, GUTMAN *et al* 1936) It is difficult to draw from these data conclusions concerning the NCE of the plasma proteins because the concomitant changes in the concentrations of phosphate sulphate and organic acids introduce an error which in some cases becomes of the same magnitude as NCE itself The data obtained for diseases in which only the proteins show pathological changes

After LOEB (1922) introduced the concept of the Donnan equilibrium in biology a large number of investigators have determined the distribution of biologically important ions across all sorts of living and artificial membranes.

Originally it was thought that this phenomenon could explain the movement of the electrolytes through the membranes but it soon became apparent that the Donnan distribution could only function as a transport mechanism if another mechanism created a condition of constant dis-equilibrium. Furthermore the Donnan distribution could not explain the transport of equally charged ions in opposite directions. As result clinical interest in the Donnan equilibrium flagged. Recently interest has returned, one of the reasons being that it provides the means to determine to what extent filtrable ions are complex-bound by the macro-molecular substances present in cartilage and connective tissue (see for instance FARBER & SCHUBERT 1957).

DISTRIBUTION OF THE MAIN FILTRABLE IONS BETWEEN PLASMA (OR SERUM) AND NATURAL (OR SYNTHETIC) INTERSTITIAL FLUID

Selection from data reported in the literature

For our purpose only results obtained with plasma or serum are of interest. A further selection had to be made with regard to the membrane which has been studied. The results obtained from the study of special compartments of the interstitial space (cerebrospinal fluid, intraocular fluid, synovial fluid) will not be considered because of the possibility or even certainty (cerebrospinal fluid), that the separating membrane is not really inert with regard to the passage of the various ions. The reader is referred to MANERY's admirable review on water and electrolyte metabolism (1954), in which the author presents and critically discusses the available data concerning the concentration ratios between plasma and other body fluids. Here we will limit oursel es to experiments in which ion distribution has been studied *in vitro* by subjecting normal plasma or serum to equilibrium dialysis or to filtration under pressure, and to *in vivo* studies concerning the distribution of the ions between serum (generally obtained from venous forearm blood) and transudates (*i.e.* edema, pleural fluid or peritoneal fluid). The results of both groups of studies are summarised in table 4 Since we are discussing in this section the findings with *normal* plasma or serum the results obtained in the clinical studies are in fact somewhat out of place here. They refer to patients in whom the plasma albumin concentration must have been rather low The authors have generally not determined the protein composition and it is therefore not possible to use these data for a study of the effect of changes in protein composition on the magnitude of the concentration ratios. Since the *difference* between the protein concentrations on both sides of the semipermeable membrane is the decisive factor in the distribution of the ions, we have followed Manery in excluding from our review those observations in which the transudate contained *more than 10* g/L of protein But even so the difference between the protein concentration on both sides of the membrane is in the clinical studies considerably less than in the *in vitro* experiments. This, together with the fact that in the clinical studies hypalbuminaemia must have been present in most cases, leads one to expect that the concentration ratios observed in the clinical studies must on the average ha been higher than those to be expected for the equilibrium between *normal* plasma and *normal* interstitial fluid. Notwithstanding these objections we have included the clinical observations in our review because they *still* provide information concerning the relative magnitudes of the concentration ratios of the different ions But the results

Gutman *et al* no data are available from which a definite conclusion can be reached as to the NCE of the plasma proteins in various diseases.

DISTRIBUTION OF THE MAIN FILTRABLE IONS BETWEEN NORMAL PLASMA OR SERUM AND (NATURAL OR SYNTHETIC) INTERSTITIAL FLUID AND CONCLUSIONS CONCERNING THE INTERACTION OF THESE IONS WITH NORMAL PLASMA PROTEINS

In the case of plasma (and serum) the study of the effect of protein on the ability of the filtrable ions to pass a semipermeable membrane has provided much information on ion protein interaction RONA & TAKAHASHI (1911) dialysed serum from a number of animals against various solutions containing 0 9 / NaCl but different concentrations of $CaCl_2$ It was assumed that the salt solution in which the Ca concentration had not changed after this compensation dialysis contained Ca in a concentration equal to the concentration of freely filtrable Ca in the serum From these experiments Rona and Takahashi concluded that about 25–35 / of serum Ca is prevented from passing through a semipermeable membrane because it has formed a complex with serum proteins. A large number of studies has since been published in which the same criterium was used to determine whether a filtrable ion was bound by the plasma proteins. All authors agreed that a considerable proportion of serum Ca was not free to filtrate and CUSHNY (1920) and TSCHIMBER *et al* (1924) found the same for Mg Within the (large) error of analysis the Na and Cl in serum appeared to be freely filtrable (VAN CREVELD 1921 RICHTER-QUITTNER 1922, NEUHAUSEN & PINCUS 1923 TSCHIMBER *et al* 1924) and RONA & PETOW (1923) concluded from dialysis experiments in neutral and acid solutions that about 14 / of serum Na was associated with proteins in a salt-type manner For K the evidence was conflicting RICHTER-QUITTNER (1922) found about 60 / of serum K to be non filtrable RONA & PETOW (1923) found a smaller but not negligible quantity of non-dialysable K but NEUHAUSEN & PINCUS (1923) concluded that all serum K was filtrable. However in the early experiments factors such as the lack of refined analytical methods, neglect of pH influence and neglect of correction for volume occupied by proteins all operated to preclude an accurate interpretation of the observed behaviour of the univalent cations Moreover the Donnan equilibrium as a cause for unequal distribution of filtrable ions was not fully appreciated until some years later

Yet presumably the best method at present available for studying ion protein interaction in a complicated solution such as plasma is to determine the concentrations of the various filtrable ions both in plasma (or serum) and its filtrate (or dialysate) and to compare the concentration ratios observed with the Donnan ratio expected The principle has been extensively used by NORTHROP & KUNITZ in the study of ion-gelatin interaction and has already been explained in chapter I If the Donnan ratio is known the amount of ion bound in a complex type manner can be calculated If the Donnan ratio is not known at least a qualitative estimate of ion-protein interaction can be made by comparing the concentration ratios of the various ions. A study of the literature concerning the distribution of ions across semipermeable membranes separating plasma or serum from a protein free filtrate or dialysate can therefore provide information concerning the nature of ion-protein interaction in plasma.

In the second part of table 4 the results of the *in vivo* studies are summarised. It has already been pointed out that these have only a relative value for the study of conditions in normal plasma. A further restriction must be made with regard to the values obtained for R_{Cl} and R_{HCO_3}. In the case of these two ions the concentration differs significantly according to whether serum from arterial or from (cubital) venous blood is analysed. It is still a matter of conjecture which of the two is most like the blood that is in equilibrium with the transudate. Both DARROW *et al* and GILLIGAN *et al* took arterial and venous blood samples. They found that the analysis of arterial blood resulted for R_{Cl} in a value about 0.5% higher but for R_{HCO_3} in a value about 5-10% lower than that obtained when venous blood was analysed. Gilligan *et al* supposed that the mean of the two values is near the true value for the equilibrium between blood and transudate.

We must conclude that in the *in vivo* studies in which venous blood was sampled the values obtained for R_{HCO_3} are definitely too high when compared with the values of R obtained for the other ions. For R_{Cl} the error is negative but so small as to be negligible in comparison to all other errors made.

Discussion of data presented in table 4

The low values found for the concentration ratio in the case of the bivalent cations and the high value found in the case of inorganic phosphate differ significantly from the ratios found for the univalent ions. As to the latter there appears to be a distinct difference between the values found for R_{Cl} and R_{HCO_3} and those found for the concentration ratios of univalent cations. In the case of the latter the ratio is consistently somewhat lower than in the case of the univalent anions.

From table 4 it is evident that for normal plasma (at normal pH) R_{Cl} is the most constant of the ratios and on the average between 0.97 and 0.98 both in the *in vitro* and in the *in vivo* studies. For R_{HCO_3} the range of reported values is considerably larger. The average value is between 0.99 and 1.00, but only two *in vitro* studies are available and since in the *in vivo* studies generally venous blood was analysed, it must be doubted whether the difference with R_{Cl} is real. The small number of values reported for the concentration ratio of inorganic phosphate are all higher than 1.0.

As to the cations in the case of Na and K the interpretation of the data is made difficult by the fact that in the early *in vitro* experiments and all clinical studies a gravimetric method of analysis was used. But at the same time the earlier studies were mostly concerned with animal sera, the later studies with human serum. From table 4 it can be seen that in the earlier studies a lower value was found for R_{K} than in the later experiments in which a flame photometer was used. Whatever the explanation may be, for Na the average value of the concentration ratio was about 0.94 in the *in vitro* studies on human serum, although the most recent publication (SALMINEN 1961) reports a somewhat lower value. The values obtained with animal sera are on the whole considerably lower with the exception of the values found by SWAN *et al* (1956), who studied dogs, and those found by DAVSON *et al* (1949) and DAVSON (1955), who studied rabbits cats and dogs. Whereas Swan used flame photometry Davson used a gravimetric Na determination as well as measurement of ^{24}Na. In the *in vivo* studies only chemical methods of analysis were used and R_{K} was on the average 0.95. At least part of the difference with those *in vitro* studies in which also a gravimetric method of Na determina-

obtained in the *in vitro* experiments are undoubtedly nearer the values to be expected for normal plasma or serum.

Manner of presenting the data in table 4

In order to make the concentration ratios comparable with the Donnan ratio – as it is usually expressed – the concentration ratios are in the case of the cations obtained by dividing the outside concentration by the inside concentration in the case of the anions by dividing inside by outside concentrations. For the bivalent ions the concentration ratio *per se* is given and not the square root of the concentration ratio

The square root derives from the theory of membrane equilibria, which is concerned with activities. A considerable quantity of bivalent cations is protein-bound in a complex-type manner and their concentration ratios are therefore certainly not equal to their activity ratios. As a consequence the use of the square root is not correct. Apparently this has not always been realised (see for instance MANERY 1954)

In table 4 the *in vitro* studies are mentioned first. The data were obtained either by equilibrium dialysis of serum against artificial interstitial fluid or by filtering serum under pressure and comparing concentrations in the ultrafiltrate with those in the serum With the second method no real equilibrium is established since the protein concentration increases continuously during the production of the filtrate. INGRAHAM *et al* (1932) have therefore used the average of the concentration in the original serum and the concen tration in the residue for the calculation of the inside concentrations. TARAIL *et al* (1952) used the original serum concentrations. It is therefore to be expected that the concentration ratios reported by Tarail *et al* will be higher than those reported by Ingraham *et al*

In the experiments of GREENE & POWER (1931) an ingenious method of *in vivo* dialysis was used In both legs the femoral artery and vein were connected by a tube of semipermeable material (not specified) with a sac on the outside containing a modified Ringer solution By using on one side a slightly hypotonic and on the other side a slightly hypertonic solution it could be seen when equilibrium was reached The concentration then found in the artificial interstitial fluid was compared with the concentration in serum obtained from femoral vein blood

In most of the studies presented in table 4 Na and K were analysed by chemical methods In the studies in which Na and K were determined by flame photometry serum or plasma was analysed The pH was about 7 40 in all studies except in that of Ingraham *et al* who experimented at pH ± 7 00 and ± 7 70 Greene and Power mention that in experiments with *in vitro* dialysis considerable changes in the concentration ratios of Cl and Na were observed when the pH changed from 6 2 to 8 2, but no details are given

The studies included in table 4 were selected because they contain data concerning the distribution of univalent ions But the number of studies in which at the same time the concentration ratios of the Ca and the Mg ions were determined is so small that we have included in table 4 the data of WALSER (1961) which are the most recent ones concerning the bivalent cations.

Rc and *R* were calculated by us from the data gi en in T ble I of Walser s publication on the assumption that water content of normal plasma is (990–0.8 P) g per litre, P indicating g protei per litre plasma (VAN SLYKE *et al* 1923 GREENE & POWER 1931). The pH alue at 37 C was calculated with the aid of Rosenthal's correction factor for temperature effect (see chapter III)

In the second part of table 4 the results of the *in vivo* studies are summarised. It has already been pointed out that these have only a relative value for the study of conditions in normal plasma. A further restriction must be made with regard to the values obtained for R_{Cl} and R_{HCO_3}. In the case of these two ions the concentration differs significantly according to whether serum from arterial or from (cubital) venous blood is analysed. It is still a matter of conjecture which of the two is most like the blood that is in equilibrium with the transsudate. Both DARROW *et al* and GILLIGAN *et al* took arterial and venous blood samples. They found that the analysis of arterial blood resulted for R_{Cl} in a value about 0.5 % higher but for R_{HCO_3} in a value about 5–10 % lower than that obtained when venous blood was analysed. Gilligan *et al* supposed that the mean of the two values is near the true value for the equilibrium between blood and transsudate.

We must conclude that in the *in vivo* studies in which venous blood was sampled, the values obtained for R_{HCO_3} are definitely too high when compared with the values of R obtained for the other ions. For R_{Cl} the error is negative, but so small as to be negligible in comparison to all other errors made.

Discussion of data presented in table 4

The low values found for the concentration ratio in the case of the bivalent cations and the high value found in the case of inorganic phosphate differ significantly from the ratios found for the univalent ions. As to the latter there appears to be a distinct difference between the values found for R_{Cl} and R_{HCO_3} and those found for the concentration ratios of univalent cations. In the case of the latter the ratio is consistently somewhat lower than in the case of the univalent anions.

From table 4 it is evident that for normal plasma (at normal pH) R_{Cl} is the most constant of the ratios and on the average between 0.97 and 0.98 both in the *in vitro* and in the *in vivo* studies. For R_{HCO_3} the range of reported values is considerably larger. The average value is between 0.99 and 1.00 but only two *in vitro* studies are available and since in the *in vivo* studies generally venous blood was analysed, it must be doubted whether the difference with R_{Cl} is real. The small number of values reported for the concentration ratio of inorganic phosphate are all higher than 1.0.

As to the cations in the case of Na and K the interpretation of the data is made difficult by the fact that in the early *in vitro* experiments and all clinical studies a gravimetric method of analysis was used. But at the same time the earlier studies were mostly concerned with animal sera, the later studies with human serum. From table 4 it can be seen that in the earlier studies a lower value was found for R_{K} than in the later experiments in which a flame photometer was used. Whatever the explanation may be for Na the average value of the concentration ratio was about 0.94 in the *in vitro* studies on human serum, although the most recent publication (SALMINEN 1961) reports a somewhat lower value. The values obtained with animal sera are on the whole considerably lower with the exception of the values found by SWAN *et al* (1956), who studied dogs, and those found by DAVSON *et al* (1949) and DAVSON (1955), who studied rabbits, cats and dogs. Whereas Swan used flame photometry Davson used a gravimetric Na determination as well as measurement of ^{24}Na. In the *in vivo* studies only chemical methods of analysis were used and R_{Na} was on the average 0.95. At least part of the difference with those *in vitro* studies in which also a gravimetric method of Na determina-

tion was used can be explained by the lower protein concentrations prevalent in the *in vivo* studies

For R_K the earlier *in vitro* studies report remarkably low values, but in the studies in which the flame photometer was used R_K was found to be about the same as R_{Na}. Notable exceptions are the lower ratios reported by FOLK *et al* (1948) and by SALMINEN (1961) From their data no explanation can be found for this deviating value Indeed both TARAIL *et al* (1952) and FOLK *et al* (1948) have commented on the fact that in the course of time the ratios reported for K have gradually risen and they conclude that the old values must have been the result of technical errors, a conclusion in which they are joined by MANERY

As was to be expected, the lowest ratios are found for Ca Those reported for Mg though somewhat higher are of the same order of magnitude. The fact that R_{Ca} was found considerably higher in the *in vivo* studies than in the *in vitro* experiments is presumably due to the differences in protein concentration and composition between the two series of observations

Concerning the effect of pH on the magnitude of the concentration ratios, only the observations of Ingraham *et al* are available. They suggest that a – physiologically speaking – large change in pH apparently has a rather small effect on R_{Cl} and R_K

THE DONNAN RATIO FOR THE EQUILIBRIUM BETWEEN PLASMA AND INTERSTITIAL FLUID

The data compiled in table 4 suggest that the differences in the values obtained for the concentration ratios of the various ions are real This makes it more difficult to make an accurate estimate of the value of the Donnan ratio The inaccuracy to be expected in using pH measurement for determining the Donnan ratio was already pointed out in chapter I and the H^+-ion concentration ratio found by HASTINGS *et al* (1927) cannot seriously be considered as representing the Donnan ratio for normal plasma. To our knowledge no further values have been published As mentioned in chapter I SALMINEN (1961) has determined the membrane potential in his ultrafiltration studies. But the average value which he found for normal serum (*i e* 9 3 mV) corresponds with a Donnan ratio of about 0 69 a value which is clearly impossible in view of the magnitude of the concentration ratios reported for Na and for Cl

An indirect estimate of the Donnan ratio has been made by VAN SLYKE WU & MCLEAN (1923) who derived an equation by which the Donnan ratio could be calculated from the sum concentration of the filtrable anions and from the base binding power of the proteins on both sides of the semipermeable membrane If no protein is present in the filtrate or dialysate the equation simplifies to (for historical reasons we have used the notation of Van Slyke *et al*)

$$r_f = \sqrt{\frac{[A]_s}{[A]_s + [BP]_s}}$$

where $[A]_s$ is the sum concentration in serum of all anions other than protein and $[BP]_s$ is NCE of proteins. Assuming with GAMBLE (1950) that for normal plasma at pH 7 40 $[A]_s = 150$ mEq/Kg H_2O and $[BP]_s = 17$ mEq/Kg H_2O we obtain for r a value of 0 949 In chapter I we have derived a similar equation and there we pointed out the

difficulties to be expected when the equation is used without due attention to ion activities. But since we are here engaged in a historical survey we will restrict ourselves to pointing out that according to Van Slyke *et al* the Donnan ratio at the capillary wall must be about 0.950 under normal conditions. This estimate is based on the assumption that normally the interstitial fluid contains negligible amounts of non filtrable ionic substances.

AMOUNT OF ION PROTEIN BOUND IN A COMPLEX TYPE MANNER IN NORMAL PLASMA

With the above estimate of the value of r in mind, the results represented in table 4 appear to indicate that in normal plasma small amounts of Cl and of HCO_3 (about 2–3 %) are bound in a complex type manner by the plasma proteins. For inorganic phosphate this amount appears to be about 15 %. When we consider the values obtained with the aid of the flame photometer the activity of the Na-ions appears to be only slightly decreased by plasma proteins, that of K-ions somewhat more. The Ca-ions show a decrease of about 30–40 % in their activity which is in fair agreement with the results obtained with the frog heart method (McLean & Hastings 1935?) and the blood coagulation method (Soulier & Crosnier 1958). In the studies reported in table 4 R_{Mg} has seldom been determined. The most one can say is that according to table 4 the activity of Mg-ions is somewhat less decreased by plasma protein than the activity of the Ca-ions.

For the univalent ions the above conclusions are compatible with the data obtained in experiments with purified albumin (see chapter I). Greene & Power (1931) calculated from their *in vivo* dialysis experiments that about 7 % of serum Na and 20 % of serum K was not dissociated, but as the Cl-ratio was assumed to indicate the Donnan ratio and the concentration ratios for Na and K are about the lowest reported, their conclusion appears to be in error. Actually in experiments with dogs, in which ascites fluid was compared with serum, Greene *et al* (1931) found both for Na and K a ratio of 0.94 (table 4) at a serum protein concentration only 20 % lower than in the *in vivo* dialysis experiments. However the studies of Greene and Power concerned dog serum and since they have been done in a very careful manner their results have to be seriously considered. Table 4 demonstrates that there are no recent *in vitro* studies comparable in scope to the studies of Hastings *et al* and Greene & Power. Furthermore only in the studies in which the flame photometer was used a ratio of about 0.94 was found for Na, and in the study by Swan *et al* the Na and K ratios were, so to speak, by-products of an investigation of the distribution of sulphate ions. In view of the fact that the normal Na concentration of plasma is still a matter of debate as a result of the very different values found by authors who all used flame photometer analysis, confirmation of the findings of Tarail *et al*, Swan *et al* and Salminen is needed before the results of the earlier investigators can be disregarded. Especially the observation that the flame photometer gives different values for Na and K concentration according to whether plasma or deproteinised plasma is analysed has serious consequences for the interpretation of the concentration ratios and the calculation of protein-bound ion.

tion was used can be explained by the lower protein concentrations prevalent in the *in vivo* studies

For R_K the earlier *in vitro* studies report remarkably low values, but in the studies in which the flame photometer was used R_K was found to be about the same as R_{Na}. Notable exceptions are the lower ratios reported by FOLK *et al* (1948) and by SALMINEN (1961) From their data no explanation can be found for this deviating value. Indeed, both TARAIL *et al* (1952) and FOLK *et al* (1948) have commented on the fact that in the course of time the ratios reported for K have gradually risen and they conclude that the old values must have been the result of technical errors, a conclusion in which they are joined by MANERY

As was to be expected the lowest ratios are found for Ca. Those reported for Mg, though somewhat higher are of the same order of magnitude. The fact that R_{Ca} was found considerably higher in the *in vivo* studies than in the *in vitro* experiments is presumably due to the differences in protein concentration and composition between the two series of observations

Concerning the effect of pH on the magnitude of the concentration ratios only the observations of Ingraham *et al* are available They suggest that a – physiologically speaking – large change in pH apparently has a rather small effect on R_{Cl} and R_{Na}

THE DONNAN RATIO FOR THE EQUILIBRIUM BETWEEN PLASMA AND INTERSTITIAL FLUID

The data compiled in table 4 suggest that the differences in the values obtained for the concentration ratios of the various ions are real This makes it more difficult to make an accurate estimate of the value of the Donnan ratio The inaccuracy to be expected in using pH measurement for determining the Donnan ratio was already pointed out in chapter I and the H^+-ion concentration ratio found by HASTINGS *et al* (1927) cannot seriously be considered as representing the Donnan ratio for normal plasma To our knowledge no further values have been published As mentioned in chapter I SALMINEN (1961) has determined the membrane potential in his ultrafiltration studies But the average value which he found for normal serum (*i.e* 9 3 mV) corresponds with a Donnan ratio of about 0 69 a value which is clearly impossible in view of the magnitude of the concentration ratios reported for Na and for Cl

An indirect estimate of the Donnan ratio has been made by VAN SLYKE, WU & McLEAN (1923) who derived an equation by which the Donnan ratio could be calculated from the sum concentration of the filtrable anions and from the base binding power of the proteins on both sides of the semipermeable membrane If no protein is present in the filtrate or dialysate the equation simplifies to (for historical reasons we have used the notation of Van Slyke *et al*)

$$r_{at} = \sqrt{\frac{[A]_s}{[A]_s + [BP]_s}}$$

where $[A]_s$ is the sum concentration in serum of all anions other than protein and $[BP]_s$ is NCE of proteins Assuming with GAMBLE (1950) that for normal plasma at pH 7 40 $[A] = 150$ mEq/Kg H_2O and $[BP]_s = 17$ mEq/Kg H_2O we obtain for r a value of 0 949 In chapter I we have derived a similar equation and there we pointed out the

More recently PRASAD and coworkers have studied ultrafiltrable Ca in various disease states including acidosis and alkalosis. A review of their work was given by PRASAD in 1960. Similar but less comprehensive studies were published by FANCONI & ROSE (1958) TERÉPKA *et al* (1958) and VAN LEEUWEN *et al* (1961). The general gist of these studies is that albumin has the highest affinity that normal γ-globulins have a lower affinity and that pathological γ-globulins show a distinctly lower tendency to form a complex association with Ca. As to the influence of pH fairly large changes are necessary before appreciable changes occur in the amount of Ca associated with protein (HOPKINS *et al* 1952, TORIBARA *et al.* 1957 LOKEN *et al* 1960, PETERSON *et al* 1961 WALSER 1962) As in the present study *our interest is mainly in* NCE *and in the univalent cations*, we shall not attempt to discuss all these data, but one further study concerning Ca must be mentioned. GLUECK *et al* (1962) observed in two patients, one with multiple myeloma and the other with an unexplained dysproteinaemia, a peculiar syndrome characterised by hyperglobulinaemia, clotting defects and osteoporosis. The most interesting finding was that, whereas total serum Ca was practically normal, ultrafiltrable Ca was definitely too low and they could show that in these patients a pathological globulin was present which bound Ca excessively

For Mg the data of PRASAD *et al* (1961) indicate that albumin shows again the highest affinity As to the other protein fractions no definite conclusions are possible from their work and no other data appear to exist. A rather peculiar phenomenon was reported by some authors, who found for Mg a very low ultrafiltrable fraction in patients with hyperthyreoidism but a very high filtrable fraction in myxoedema (SOFFER *et al* 1939 1941 and DOVIE & LAVIETES 1942). Their observations could not be confirmed by COPE & WOLFF (1942) and BISSELL (1945), and from the discussions by Soffer and by Dovie it does not become clear how the observed phenomenon must be explained. The total Mg concentrations were not significantly different from normal and no data are given about protein composition and plasma lipids.

From this short survey it is evident that more observations and experiments are necessary to obtain accurate information concerning the interaction between protein and filtrable plasma ions in disease. Even in the case of Ca there is still need for a systematic study of plasma or serum containing pathological proteins. The observation of Glueck *et al* should be a warning that in certain diseases proteins with unexpected affinity for cations (or anions) may circulate.



Summary of chapter II

The experiments with horse serum published in 1928 by Van Slyke *et al* represent still the most recent determination of the *NCE of plasma* proteins. According to it the value of NCE (at pH 7.35) is 23.5 mEq/100 g protein, or about 16 0 mEq/L for normal plasma.

There are objections against applying to human plasma the results obtained by Van

In conclusion the following can be said An accurate estimate of the Donnan ratio (for normal serum or plasma) is not available But if we accept a value of 0 950 for the *in vitro* experiments listed in table 4 the differences between the concentration ratios found for the various filtrable plasma ions apparently indicate the existence of a complex type of interaction between Ca, Mg, Cl phosphate and presumably also HCO_3 on the one hand, and the plasma proteins on the other In the case of Na and K the evidence is conflicting.

It would appear that further experiments are needed concerning the concentration ratio of the univalent cations and anions under conditions which are well defined as to protein concentration pH, ionic strength and electrolyte composition These experiments should also include a study of the relation between pH and concentration ratio as such data are extremely scarce

INTERACTION OF FILTRABLE IONS WITH PLASMA PROTEINS UNDER PATHOLOGICAL CONDITIONS

The number of studies concerning the univalent ions is very small indeed and limited to observations on the concentration ratio of Na and/or K in ultrafiltration experiments. Only in two of these studies both cations were analysed TARAIL *et al* (1952) found a normal value for the concentration ratios when they averaged the results obtained with serum from 10 patients (among whom 4 with renal insufficiency and 4 with diabetes mellitus) But the range of values was considerably larger than in normal persons and the protein content and the protein composition are not reported SALMINEN (1961) studied the serum of a much larger number of patients (suffering from hepatic cirrhosis, nephrosis pulmonary tuberculosis or renal insufficiency) and determined among other things both the protein concentration and the protein composition His main conclusion is that the albumin concentration of the serum is the most important factor determining the concentration ratios of Na and K Ultrafiltration experiments with solutions containing purified protein fractions showed that at pH 7 40 γ globulin does practically not affect the concentration ratios One may conclude from his experiments that whereas at pH 7 40 albumin binds fairly large amounts of both Na and K – presumably in a salt type manner – γ-globulin has no affinity for these cations at physiological pH values.

More is known concerning the interactions between the serum proteins and the bivalent cations, in particular Ca One of the earliest studies in patients was done by BENDIEN & SNAPPER (1933) who from their data concluded that Ca interacted with albumin only Later studies proved that although most of the non filtrable fraction of serum Ca is indeed associated with albumin, some is also bound by the globulin fraction and GUTMAN & GUTMAN (1937) already suspected that differences in affinity for Ca existed within the group of globulins CHU & HASTINGS (1938) concluded from *in vitro* dialysis experiments that the normal globulins have a greater affinity for Ca than the pathological euglobulin found in the diseases studied by Gutman and Gutman, *i e* multiple myeloma and lymphogranuloma inguinale DRINKER *et al* (1939) using the frog heart method found that the globulin fractions which precipitate at the lowest salt concentration show a much smaller affinity for Ca than the fractions which precipitate at higher salt concentrations

PART II

Slyke *et al* which make it necessary to determine NCE in human plasma. The values reported for the ionic composition of normal human plasma were found no help in this respect, both because of the limited number of studies from which a reasonable estimate of NCE can be made (assuming the concentration of the rest anions 6 0 mEq/L in normal plasma) and because the estimates thus obtained vary from 10 to 25 mEq/L. This variation is apparently largely the result of the spread in the values reported for normal plasma Na concentration a spread which is not easily explained

Concerning the value of NCE in disease, accurate quantitative data do not exist, and reliable estimates from the ionic composition of plasma are very scarce indeed.

As to the concentration ratios of the filtrable ions for the equilibrium between normal *plasma (or serum) and natural (or synthetic) interstitial fluid, the number of studies is* surprisingly small and the values obtained show a considerable spread. But it would appear that there is a real difference between the average ratios found for the univalent cations and those found for the univalent anions, suggesting that either or both are partially bound in a complex type manner A definite conclusion is not possible since at present no accurate values are known for the Donnan ratio Also data concerning the correlation between the pH and the concentration ratios of the plasma ions are virtually non-existent.

Concerning the protein binding of univalent ions in disease practically nothing is known in contrast to the numerous data which have been gathered with regard to the protein binding of Ca

CHAPTER III

Chemical analysis

The method adopted for the determination of the net cation equivalency of plasma proteins (chapter IV and chapter V) is rather susceptible to errors in analysis, since the results of at least seven different methods cumulate in the final value For protein Na, Cl and HCO_3 in particular a relatively small percentage error in analysis will result in a large error in NCE. Special attention will therefore be given to systematic and accidental errors in general and to those in the case of protein Na, Cl and HCO_3 in particular

SYRINGES, GLASSWARE AND STOPPERS

Dry Luer Lok syringes were used throughout. After soaking in warm tap water they were brushed, vigorously rinsed with tap water boiled for 20 minutes in distilled water and left to dry i sterile containers.

All glassware was kept separated from that used for routine determinations. After soaking in water and brushi g, the glass tubes were stored in potassium dichromate sulfuric acid solution for at least 24 hours, then thoroughly rinsed with tap water and with distilled water and finally dried in a sterilizer

For closure of the glass tubes rubber caps and rubber stoppers were used. After soaking and brushing they were boiled in distilled water rinsed and dried.

An isotonic NaCl solution stored for 24 hours in stoppered glass tubes, cleaned in the manner described, failed to show any change in Na and Cl concentration.

OBTAINING AND HANDLING OF SAMPLES

In **ivo ultrafiltration**

The samples were obtained from a cubital vein via an indwelling 18 *gauge needle, which* between sampling was fitted with a stylet. 14–18 ml blood was drawn in 10 *ml syringes* nd immediately injected through a rubber cap into a round-bottom glass tube flushed w th end-expiratory air just prior to the beginning of the experiment The capacity of the tubes varied from 16 to 20 ml. Until 29-5-61 the tubes contained 10 µl of a 5 / heparin solution. Then quite suddenly late *coagulation* started to occur in some of the plasma samples and from 8-6-61 on the amount of heparin was *increased to* 30 µl

According to the manufactures no change in the fabrication procedure had been made and the altered effectivity of the heparin solution may have been due to the ageing of batch.

Immediately after sampling the blood samples were placed for 4 minutes in a water bath with the indicated temperature so that they had the correct temperature before they were put into the thermostatted centrifuges. The samples to be compared were centrifuged simultaneously. During centrifugation the temperature of the blood remained constant to within 1°C.

Loss of CO_2 – It is highly improbable that the 0.15 ml air contained in the dead space of the 10 ml syringes induced CO_2 loss during sampling, since drawing 2 ml air into a syringe containing 6 ml blood and subsequent gentle shaking did not alter the pH of the blood significantly. Nor did lowering the pressure in the syringe to about 400 mm Hg (table 8). This remarkable resistence to CO_2 loss may be partly explained by the fact that the blood cools on entering the syringe, which increases the solubility of the gas.

Flushing the rubber-capped test tubes with end-expiratory air was presumably adequate in preventing loss of CO_2 since no change in pH and only a slight change in Hb saturation were observed during the transfer of blood from the syringe and the subsequent tilting necessary to mix heparin and blood (table 9).

We conclude that the plasma on which the determinations were done represents 'true' plasma but for the addition of the heparin solution. A correction can be made for the latter (see chapter VI).

Equilibrium dialysis

10 ml Luer Lok syringes were used. First 1 ml of the sample was drawn with which the air of the dead space was expelled, and then the rest of the sample – 10 ml – was drawn. About 6–7 ml was injected into rubber stoppered test tubes, 3 ml remaining in the metal-capped syringe for determination of pH and total CO_2. The pH of serum and of a bicarbonate buffered saline solution did not alter during 12 hours storage in the syringes. All chemical determinations were done within 7 hours after sample taking.

CHEMICAL METHODS

All chemical determinations were done in duplicate, unless stated differently.

Water content

Technique

Glass stoppered weighing bottles were used (diameter 25 mm, height 25 mm). After cleaning they were stored in potassium dichromate sulfuric acid solution and before use thoroughly rinsed with distilled water.

After placing the weighing bottles in an electric steriliser at 108°C, constant weight was reached within 24 hours. Then a pipet, accurately calibrated to contain a volume of the order of 0.5 ml, was filled with the fluid to be investigated. The pipet was weighed before and after filling, the difference representing the wet weight of the sample. The latter was then delivered into the weighing bottle, several washings of distilled water being used to effect a complete transfer (by following this procedure both water content and the specific gravity of the sample could be calculated). The bottles were then placed again into the steriliser and 24 hours later they were reweighed after cooling to room temperature in an exsiccator. The difference between this weight and the weight of the bottle found before introduction of the sample represents the dry weight of the sample.

In the first experiments 3 ml blood of each sample was injected separately into a small rubber capped glass tube (containing 5 µl 5 % heparin solution and end-expiratory air) for the determination of total CO_2. We repeatedly tested CO_2 content and Cl concentration of both the small and the larger tube. They were always found to be identical within the errors of determination. A representative example is given in table 5. In later experiments the total sample was injected into the larger tube and after centrifugation 2 ml plasma was drawn anaerobically for CO_2 determination into 2 ml Luer Lok syringes which were closed with a metal cap after expelling the air contained in the dead space of the syringe. No loss of CO_2 could be detected during this procedure (table 6).

Within ten minutes after the blood samples were drawn the blood was centrifuged (8 min. 3000 rpm. at 22–24°C) and the plasma pipetted into dry rubber stoppered test tubes (in the later experiments after a sample was separated for total CO_2 determination in the manner described above).

Blood for determination of pH and percentage HbO_2 was drawn immediately after the large sample in a 5 ml Luer Lok syringe, the dead space of which (±0.1 ml) was filled with 5 % heparin solution. The metal capped syringes were placed in ice water.

All chemical determinations were done within 7 hours after sampling, but pH and HbO_2 were measured within 2 hours.

Changes in the composition of plasma caused by the sampling and centrifugation of blood and the subsequent handling of plasma

THE ADDITION OF HEPARIN SOLUTION. – We used Thromboliquine Organon which contains 5 % heparin and the following amounts of ion (in mEq/L): Na 320–340, K 3–13, Cl 120–135, no Ca or Mg (3 phials from 2 different batches). The errors resulting from the addition of the heparin solution are small and will be discussed when the results of the *in vivo* ultrafiltration experiments are presented (chapter VI).

HAEMOLYSIS. This occurred only exceptionally and in the course of our experiments not more than 10 samples had to be rejected for this reason. When the aspect of a plasma sample was dubious its K concentration was used for a guide: when the latter was in keeping with the K concentrations found in the samples immediately preceding and following the suspected sample, the latter was accepted.

EFFECT OF TEMPERATURE DURING CENTRIFUGATION. – ROSENTHAL (1948) observed a lower total CO_2 content and Cl concentration when plasma was obtained by centrifuging at room temperature instead of at 38°C. No experimental data are given. ASTRUP (1956) reports one experiment in which he observed a 2 % decrease in plasma CO_2 content when erythrocytes and plasma were separated at room temperature instead of at 38 C. C. MOLLER (1959) mentions that he observed a similar effect but gives no details. On the other hand SEVERINGHAUS *et al.* (1956[1]) in well documented experiments found no difference in plasma CO_2 content whether the blood was centrifuged at 37 C or 5 C.

We could find no significant difference in the concentrations of Na, K, Mg, Ca, Cl, total CO_2 and protein whether the blood was centrifuged at 38°C or at room temperature (22–24°C) (table 7).

Nitrogen factor

Originally we used a catalyst mixture containing selenium, but this leads to a loss of nitrogen (HILLER *et al* 1948, WILLITS *et al* 1949 1950). This became evident when with our original technique we determined the N-factor on dialysed alcohol-ether extracted bovine albumin and obtained a value of 6.60. Although most of the values published for the N-factor are well above 6.25 and SUNDERMAN *et al* (1958) – apparently using the same technique as Willits *et al* – have recently reported a value of about 6.55 for all electrophoretic fractions of serum protein, HILLER *et al* (1948) in a very careful investigation found a value of 6.22 and 6.24 for two different samples of pooled human plasma. Their macro-Kjeldahl results showed good agreement with the results obtained with the Dumas method, and their observations are so well documented that we accept their conclusion, that for plasma proteins the protein/N ratio is 6.25 after correction for non-protein nitrogen. With our present technique of Kjeldahl analysis we found a N factor of 6.32 for one sample of dialysed alcohol-ether extracted human albumin, and of 6.25 for one sample of human globulin treated in the same way. In view of the fact that in our experiments the fat extraction has been less rigorous than in those of Hiller *et al* the dry albumin may have included more lipids in our case. We conclude therefore that with our technique of N analysis the use of 6.25 as N-factor is warranted. This is supported by the fact that we found the partial specific volume for the albumin sample to be 0 738 and for the globulin sample 0 735 which agrees reasonably well with the results of the specialised measurements quoted by PHELPS & PUTNAM (1960), to wit 0 733 and 0 739 respectively

Protein (biuret method)

This method was used for the determination of protein in all our experiments.

Technique

The analysis was done according to the description of GORNALL *et al* (1949) 1.5 g $CuSO_4$ $5H_2O$ 6.0 g $KNaC_4H_4O_6$ $4H_2O$ and 300 ml 2.5 N NaOH are dissolved in distilled water to a total volume of 1 litre. In a test tube 8 ml of this reagent are mixed with ml of distilled water then 0 1 ml plasma is added and the contents of the tube thoroughly mixed again by swirling. The color is left to develop at room temperature fo 60 minutes, when it has become practically constant (less than 1 / further increase n optical density (O D) over the next 15 minutes) Then the transmittance is read at 540 mμ in an Evelyn colorimeter against a blank of 8 ml reagent and 2.1 ml distilled water Determinations are done in duplicate and the same tube is used for all colorimeter readings The transmittance is read to the nearest ½ scale unit at 60 g/L protein this corresponds to 0.3 g/L, at 100 g/L protein to 0 7 g/L

The trichloroacetic acid filtrate of fresh normal plasma was found to contain no biuret positive substances. The contribution of the plasma per se to the O D was tested with mock biuret reagent which contained all constituents with the exception of $CuSO$ Otherwise the procedure was as described above Clear to slightly opalescent plasma samples did not influence O D., distinctly icteric plasma gave slight increase only but both haemolysis and lipaemia had marked effect As mentioned already the few haemolytic samples in our experiments were rejected and since all ultrafiltration experiments are done in the fasting state some degree of lipaemia was only observed in the plasma of three patients with nephrosis In these instances the

From the difference between wet weight and dry weight and the known volume of the pipet the water content of the sample was calculated in g per litre plasma at room temperature

Samples were determined in triplicate and with each set of determinations a blank of distilled water was run also in triplicate All weighings were done on a Mettler analytical balance type H 16 which can be read to the nearest 0.01 mg

Systematic error

A 24-hours stay at 108°C was found to be sufficient since in 30 experiments no further change in weight was observed when drying was continued up to 72 hours, or vacuum extraction over P_2O_5 was carried out additionally

Furthermore the measured density of distilled water never differed by more than 0 8 g ‰ from the density to be expected at the temperature of ambient air the average value of the latter being 21 3 C (range 15 8–26 7) For a total of 121 determinations the mean difference between the two was −0.25 ‰ (extremes +0 5 to −0 8‰), the measured density being slightly lower The difference would be fully explained if the temperature of the samples was on the average 1 C higher than that of the ambient air In view of the unavoidable touching of the pipet it is concluded that there is no evidence of a significant systematic error when samples of distilled water are dried

Accidental error

From the triplicate values obtained in all *in vitro* ultrafiltration experiments the standard deviation of a single determination of water content was calculated and the standard error of the mean This was done separately for the samples containing respectively 950 to 930 929 to 910 and 909 to 840 g water per liter plasma From table 10 it can be seen that expressed in absolute values, the SE of the mean was the same for the three groups

Protein (macro-Kjeldahl method)

The macro-Kjeldahl method was used to calibrate the biuret method accepting the factor 6.25 for converting grams of nitrogen to grams of protein

Technique

This was essentially the one described by WILLITS *et al* (1949 1950) To 0 5 ml sample in a 100 ml Kjeldahl flask is added 7 ml catalyst mixture (4 g HgO 100 g Na_2SO_4 and 200 ml 96 % H_2SO_4 made up to 700 ml with distilled water) Digestion is continued for 30 minutes after the content of the flask has become clear boiling has to be vigorous. After transferring the digested sample to the Parnas Wagner apparatus 20 ml 50 NaOH + 5 ml 8 $Na_2S_2O_3$ is added and distillation started. The NH_3 is received in 10 ml 0 075 N H_2SO_4 to which a bromcresolgreenmethylred indicator is added and the excess H_2SO_4 is titrated with 0 1000 N NaOH With each series of plasma determinations a blank and a standard solution of $(NH_4)_2SO_4$ are analysed With this technique we constantly recovered 99–101 % of the nitrogen both for $(NH_4)_2SO_4$ and for tryptophan Plasma was corrected for non-protein nitrogen and duplicate determinations did not differ by more than 0.5 %.

original sample. The pipets used for making up the different dilutions were calibrated against each other so that the simulated concentrations could be calculated from the sample's original protein concentration to within 0.2 %. For all samples the protein concentrations so obtained were plotted against the optical densities measured

The resulting scatterdiagram is shown in fig. 9 main graph. It can be seen that the deviation from linearity starts at a protein concentration of about 80 g/L and that the use of a linear calibration line would underestimate the protein concentration by about 1–1.5 % at 100 g/L and by about 3 % at 140 g/L. The line drawn through the experimental points in fig. 9 main graph, is the final calibration line from which the conversion table was derived

For the precise construction of its alinear trajectory the following procedure was adopted. The relation between O D and protein concentration was considered separately for the 19 series of dilutions, and for each point within series the observed deviation from linearity was expressed as percentage of the (hypothetical) protein concentration which would have been obtained if the relation O D -protein concentration were linear throughout. Thus for each point show in the scatterdiagram of fig. 9 its 'percentage deviation from linearity' was obtained. Next, the values on the O D scale higher than 0 200 wer subdivided in units of 0 0554 (which is equivalent to 10 g/L protein on the linear part of the calibration line) and for all experimental points falling within one such 'unit' of O.D the mean percentage deviation from linearity' was calculated and plotted against the mean of their optical densities. Through the points so obtained the line of best fit was drawn. The result is shown in the small graph occupying the lower right-hand corner of fig. 9 for each group the range found for the percentage deviation from linearity is represented by horizontal bar and the number of experimental points indicated

A further indication that the relation between O D and plasma concentrations higher than 75 g/L was not linear is the following observation In 19 *in vivo* ultrafiltration experiments the protein concentration of the samples containing more than 80 g/L plasma was determined both in the (normal) final plasma dilution of 1 101 and in a final dilution of 1 201 Again it was found that for higher protein concentrations the relation between O D and protein concentration is not a linear one, and the deviation from linearity in the range 80–120 g protein/L plasma calculated from these experiments was in perfect agreement with that shown in fig. 9

The deviation from linearity was exactly the same whether O D was measured in the Evelyn colorimeter or in the Unicam SP 500 spectrophotometer and whether plasma was analysed or purified bovine albumin.

Observations made by MEHL (1945) suggest that with the method of GORNALL *et al.* a relative shortage of copper ions occurs when analysing plasma containing more than 120 g protein per litre. Originally we rejected this explanation for the shape of our calibration line because in first series of experiments no effect as observed when copper content and tartrate concentration of the biuret reagent were increased Later we learned that STRICKLAND *et al* (1961) made study of copper binding by proteins in lkaline solutions and that they observed rectilinear relation between O D and serum protein concentration, provided that the amount of protein per mole of copper ion did not exceed 400 grams. With the method of Gornall et al. the concentrations in the final reagent mixture for copper sulfate, tartrate and sodiumhydroxide are 0.0048 M/L, 0 017 M/L and 0 60 M/L respectively In theory therefore, the concentration of copper ions is sufficiently high to guarantee a calibration line lich is rectilinear up to concentration of 3 g protein per litre of the final solution, corresponding with 300 g protein per litre plasma. This is not so, and have since found that increasing the copper concentration to 0.01 M/L in the final reagent mixture / the actual concentration used by Strickland *et al.* did restore the rectilinearity of the calibration line for the protein range show in fig 9 Changes in the concentration of tartrate or of sodiumhydroxide had no effect. Clearly therefore, the shape of our cali-

O D of the plasma itself was determined, and the optical density measured with the biuret reaction corrected accordingly

Calibration line

The O D so obtained is directly converted to protein concentration with the use of a table which was constructed from the following data. Up to a protein concentration of 75 g/L the relation between O D and protein concentration was found to be linear and, from macro-Kjeldahl analyses on plasma containing 52 to 75 g/L protein, calculated as protein (g/L) = 181 0 × O D with our technique This relation was repeatedly determined during the course of our experiments and the conversion factor found varied from 179 0 to 182.0 (8 determinations) However for protein concentrations higher than 75 g/L the relation with O D becomes alinear the conversion factor increasing steadily At a concentration of 100 g/L protein the deviation from linearity is only 1–1 5 %, which presumably explains why Gornall *et al* concluded that the relation was linear up to that point. But during *in vivo* ultrafiltration the protein concentration can rise to 120 g/L – with pathological plasma even to 160 g/L.

The relation between O D and protein concentrations higher than 75 g/L was therefore determined experimentally and the extended calibration line thus obtained used for the calculation of the corresponding part of the conversion table. For these experiments 19 samples were used 1 sample of purified bovine serum albumin, 3 different

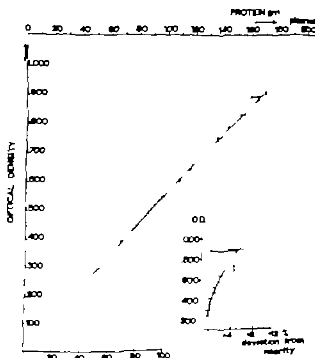


Fig. 9 Construction of calibration line used with the biuret method. See text for explanation

samples of individual plasma and 15 different samples of pooled plasma. Their protein concentrations ranged from 55 to 79 g/L and therefore fell within the linear part of the calibration line By varying for each sample the ratio between plasma and distilled water while keeping constant final volume and the concentration of the biuret reagent we simulated protein concentrations ranging from 0 15 times to 3 times the value of the

original sample. The pipets used for making up the different dilutions were calibrated against each other so that the simulated concentrations could be calculated from the sample's original protein concentration to within 0.2 %. For all samples the protein concentrations so obtained were plotted against the optical densities measured.

The resulting scatterdiagram is shown in fig. 9 main graph. It can be seen that the deviation from linearity starts at a protein concentration of about 80 g/L and that the use of a linear calibration line would underestimate the protein concentration by about 1–1.5 % at 100 g/L and by about 3 % at 140 g/L. The line drawn through the experimental points in fig. 9 main graph, is the final calibration line from which the conversion table was derived.

For the precise construction of its alinear trajectory the following procedure was adopted. The relation between O.D. and protein concentration was considered separately for the 19 series of dilutions, and for each point within a series the observed deviation from linearity was expressed as percentage of the (hypothetical) protein concentration which would have been obtained if the relation O.D.-protein concentration were linear throughout. Thus for each point shown in the scatterdiagram of fig. 9 its 'percentage deviation from linearity' was obtained. Next, the values on the O.D. scale higher than 0.200 were subdivided in units of 0.0554 (which is equivalent to 10 g/L protein on the linear part of the calibration line) and for all experimental points falling within one such 'unit' of O.D. the mean percentage deviation from linearity was calculated and plotted against the mean of their optical densities. Through the points so obtained the line of best fit was drawn. The result is shown in the small graph occupying the lower right-hand corner of fig. 9; for each group the range found for the percentage deviation from linearity is represented by a horizontal bar and the number of experimental points indicated.

A further indication that the relation between O.D. and plasma concentrations higher than 75 g/L was not linear is the following observation. In 19 *in vivo* ultrafiltration experiments the protein concentration of the samples containing more than 80 g/L plasma was determined both in the (normal) final plasma dilution of 1 : 101 and in a final dilution of 1 : 201. Again it was found that for higher protein concentrations the relation between O.D. and protein concentration is not a linear one, and the deviation from linearity in the range 80–120 g protein/L plasma calculated from these experiments was in perfect agreement with that shown in fig. 9.

The deviation from linearity was exactly the same whether O.D. was measured in the Evelyn colorimeter or in the Unicam SP 500 spectrophotometer, and whether plasma was analysed or purified bovine albumin.

Observations made by MEHL (1945) suggest that with the method of GORNALL *et al.* a relative shortage of copper ions occurs when analysing plasma containing more than 120 g protein per litre. Originally we rejected this explanation for the shape of our calibration line because in a first series of experiments no effect was observed when copper content and tartrate concentration of the biuret reagent were increased. Later we learned that STRICKLAND *et al.* (1961) made a study of copper binding by proteins in alkaline solutions and that they observed a rectilinear relation between O.D. and serum protein concentration, provided that the amount of protein per mole of copper ion did not exceed 600 gram. With the method of Gornall *et al.* the concentrations in the final reagent mixture for copper sulfate, tartrate and sodiumhydroxide are 0.0048 M/L, 0.017 M/L and 0.60 M/L respectively. In theory therefore, the concentration of copper ions is sufficiently high to guarantee a calibration line which is rectilinear up to a concentration of 3 g protein per litre of the final solution, corresponding with 300 g protein per litre plasma. This is not so, and we have since found that increasing the copper concentration to 0.01 M/L in the final reagent mixture (i.e. the actual concentration used by Strickland *et al.*) did restore the rectilinearity of the calibration line for the protein range shown in fig. 9. Changes in the concentration of tartrate or of sodiumhydroxide had no effect. Clearly therefore, the shape of our cali-

bration line is after all the result of too low a copper concentration. We have no explanation for the misleading results of our first series of experiments.

As a final test different dilutions of 4 plasma samples were analysed, both with the method of Gornall *et al* using the conversion table described above, and according to Strickland *et al* The values obtained agreed to within 0.5 ‰ even though the protein concentration in the final reagent mixture was as high as 1.8 g/L (corresponding to 180 g protein per litre plasma).

Systematic error as judged from comparison with macro-Kjeldahl

In table 11 the macro-Kjeldahl method is compared with the biuret method using the correct calibration line The values agree to within 1 / over the range 60 to 111 g/L plasma protein with the notable exception of the two samples B1 and B2, and most of the plasma samples from patients with multiple myeloma.

We have no explanation for the discrepancy observed with B1 and B2, but the patients with multiple myeoloma all had very high concentrations of myeloma protein and it is questionable whether the normal N-factor holds for these proteins It is also possible that in these proteins the relation between N content and biuret reactive groups is different from that found for the ordinary plasma proteins (MEHL *et al* 1949) but Gornall *et al* could find no difference in the case of an abnormal serum globulin (precipitated with 13 5 / Na_2SO_3 but not further specified)

It is concluded that at the protein concentrations observed in our experiments, no systematic discrepancy exists between the values obtained with the macro-Kjeldahl method and those obtained with the biuret method unless there is a high concentration of abnormal protein

Accidental error

From all the duplicate values obtained in the *in vivo* ultrafiltration experiments the standard deviation of a single determination was calculated and the standard error of the mean This was done separately for the protein concentration ranges shown in table 12. The higher the protein concentration the smaller the percentage error Throughout the protein concentration range encountered in our experiments the SE of the mean (expressed in g per litre) remains the same.

Protein fractions

We have used both a salting-out technique and paper electrophoresis

Salting-out

The method of MAJOOR (1946) was used, which discerns an albumin a pseudo-globulin and an euglobulin fraction by differentiation of solubility curves, using a 28 and a 19 disodium sulphate solution respectively The protein content of the fractions obtained is determined with the biuret method

Paper electrophoresis

This was performed on Schleicher and Schüll paper 2043 B using a barbital buffer (pH 8 6) and a voltage of 110 V After staining the proteins with *amido-black 10 B* the strips are cut, eluted with NaOH and the fractions determined quantitatively by colorimetry Technique accuracy and reproducibility of the method have been described in detail by ENNEKING (1956). However as Enneking studied sera and we have analysed

plasma as well, some remarks must be added. When plasma is analysed by paper electrophoresis, one finds a band situated between the β- and the γ-bands, which is only very vaguely seen in serum obtained from the same blood For a correct assessment of the protein composition of the plasma samples it is necessary to cut out this part of the strip separately and determine the 'fibrinogen' content by elution, subsequent colorimetry of the eluate and calculation of 'fibrinogen' by the use of an appropriate factor necessary to correct for the difference in the adsorption of colour between albumin and fibrinogen

Correction factor for fibrinogen This factor was not determined by Enneking but could be found in the following manner a concentrated solution of fraction I (cold ethanol method 6), containing 77 4 g/L protein and a solution of crystalline human albumin containing 78.5 g/L protein, were compared by pipetting 0.1 ml of each solution on paper colouring with amido-black, drying, elution and colorimetry For the eluate of fraction I an O D of 297 was found, for the albumin eluate an O D of 427 and this gives a correction factor of (427/297) (77 4/78.5) = 1 42. With paper electrophoresis the solution of fraction I was found to contain γ-globulin, 'fibrinogen' and small amounts of β- γ- and α_1-globulin. From the correction factor calculated for the solution as a whole and the correction factor found by Enneking for respectively the γ- (1 76), β- (1 40), α_2- (1.50), and α_1- (1 52) globulins, the correction factor for fibrinogen was finally calculated at 1.34 (the actual values found in three experiments were 1.30, 1.38 and 1.35). To check the correctness of these calculations a diluted solution of fraction I was added to a sample of normal plasma, and then the normal plasma, the diluted solution of fraction I and the mixture were all three analysed by electrophoresis using the above-mentioned correction factors. The result is given in table 13 and shows a reasonable recovery (106 %) for 'fibrinogen' which is at least as good as, if not better than, the recovery found for the globulin fractions.

Systematic error

When comparing his method with the moving boundary electrophoresis, Majoor found the quantity of the albumin fraction practically equal with both methods. The *euglobulin* fraction was quantitatively somewhat larger than the γ-globulin fraction (MAJOOR 1947). ENNEKING (1956) found (in serum of patients with renal disease) with paper electrophoresis the albumin fraction about 8 % lower than with the method of Majoor and the γ-globulin fraction considerably lower than the euglobulin fraction as determined with the method of Majoor

It must be concluded that, quantitatively the albumin fraction determined according to Majoor is practically equal to the electrophoretic albumin fraction, but that the euglobulin fraction contains not only γ-globulin, but also β-globulin and possibly γ-globulin The amount of β- and γ-globulin precipated as euglobulin presumably depends on the relative concentrations of the fractions (ENNEKING 1956).

Na and K

These were determined by flame photometry

Apparatus

This was constructed by Dr VRIES and is described in GORTER & DE GRAAFF (1955). It operates with two barrier-layer cells and two galvanometers, so that sodium and

potassium determination can be done *simultaneously*. No internal lithium standard used. The atomiser consists of two wide bore glass capillaries with drawn-out tip and mounted at right angles. Air is used at a pressure of 90.0 cm Hg which is kept constant within 0.5 %. The atomiser consumes about 16 ml sample per minute. The burner provided with a fine mesh and gives a stable flame. Propane gas is used at a pressure of 45 cm H_2O.

For the Na and K analysis a combination of Corning glass filters is used the characteristics of which are shown in fig. 10.

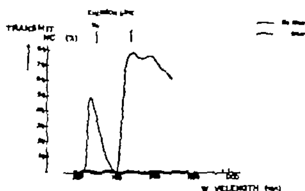


Fig. 10 – Transmittance of filters used in flame photometer

The galvanometers are mounted on a rigid support against the outer wall of the laboratory building. In the course of our studies the Na galvanometer had to be changed (at 10–12–60). The galvanometer scales were such that with the technique used and in the concentration range observed in our experiments, the smallest difference between two readings corresponded to a difference in Na concentration of respectively 0.25 mEq/L (first galvanometer) and 0.14 mEq/L (second galvanometer). The change of the galvanometer will be neglected in the presentation of our results since we found the same value for the SD of a single plasma Na determination before and after 10–12–60, and no difference in the experimental results was observed (see Addendum). For the K galvanometer the smallest difference between two readings corresponded to 0.02 mEq/L K.

The following substances were found to have no influence on the emission of an aqueous solution containing 140 mEq/L Na and 5 mEq/L K (all concentrations refer to the undiluted solutions): $CaCl_2$ (20 mEq/L), $MgCl_2$ (8 mEq/L), $CuSO_4$, $ZnSO_4$, $MnCl_2$, $Fe(NH_4)_2SO_4$, $CdSO_4$, ammoniummolybdate (each in a concentration of 0.5 mM/L), the anions SO_4 (145 mEq/L), HCO_3 (140 mEq/L) and PO_4 (100 mM/L) and the organic substances urea (6 g/L) and glucose (10 g/L). In confirmation of the literature H ions were found to influence emission since a depression of the latter was observed when the pH value of the solution sprayed into the flame was below 6.0. In the dilution used by us the pH was higher than that, unless an acid protein precipitant had been used.

Technique

The initial stages of the procedure differed according to whether plasma (or serum) was analysed directly, or after protein precipitation.

When plasma was not deproteinised we proceeded as follows. 0.5 ml plasma was injected with a calibrated syringe pipet into a 50 ml volumetric flask (Leerdam) and 1 : 100 diluted with electrolyte-free water.

When plasma was deproteinised the following procedure was used. To 0.5 ml plasma 10 ml 6 % trichloroacetic acid was added. After centrifugation 8 ml supernatant was pipetted into a 40 ml volumetric flask (Leerdam) and electrolyte-free water added to the mark. In this case the final dilution of plasma was therefore 1 : 105.

In both cases the standard solutions were treated in the same way as the plasma samples.

Four standard solutions were used containing NaCl and KCl in the following concentrations (mEq/L): 120 – 3, 130 – 4, 140 – 5 and 150 – 6. With each series of determinations a blank (electrolyte-free water) and at least three of the standard solutions were run. After each sample the standard solution nearest in concentration was re-analysed. If the galvanometer reading for Na had changed, the sample and the standard solution were redetermined until identical readings were obtained for the standard solution before and after the analysis of the sample. Furthermore, when the difference between duplicate determinations was larger than 0.5 mEq/L for Na or 0.05 mEq/L for K, both solutions were re-analysed and the first pair of determinations discarded.

In the case of deproteinised plasma (or serum) the values obtained were always corrected for the error due to the volume change resulting from the removal of protein.

> In plasma containing 70 g/L protein with a specific volume of 0.8, the protein occupies 5.6 % of the plasma volume. In the procedure for deproteinisation one part of plasma is diluted with 20 parts of 6 % trichloroacetic acid. In the final 1 : 21 dilution the protein occupies 0.26 % of the total volume. As the protein is precipitated, only 99.74 % of the original volume is left, which contains quantitatively the other solutes of the original plasma sample. Consequently their concentration increases with 0.26 %, whereas in the standard solutions, which do not contain protein, no relative increase in concentration of the solutes occurs. The necessary correction evidently depends on the degree of plasma dilution in the solution from which the protein is precipitated. For a 1 : 6 dilution the correction is – 0.9 %, for a 1 : 2 dilution – 2.8 %.
>
> The values mentioned in the following pages for deproteinised plasma or serum always refer to corrected ones, the correction having been made from the known protein content of the sample and an assumed specific volume of 0.80.

In view of the discussion of systematic errors which is to follow it is necessary to mention already here that with the technique described above the SD for a single plasma determination was 0.1 mEq/L for Na and 0.01 mEq/L for K.

Systematic error

The fact that the values published for the normal plasma Na concentration after the introduction of the flame photometer still range from about 135 to about 145 mEq/L and the fact that no common operator can be detected to explain this variation (chapter II) lead one to suspect that systematic errors exist which differ according to the apparatus and technique employed. During the preparation of this manuscript Bergström and Hultman reported that they found, both for Na and K, a lower value when plasma was analysed than when the solution obtained after protein precipitation was analysed. They concluded that the latter value was correct and that the low value obtained with plasma was due to a "protein interference effect" (see chapter II). We also found a difference between Na and K values obtained with plasma (or serum) and those obtained

potassium determination can be done simultaneously. No internal lithium standard is used. The atomiser consists of two wide-bore glass capillaries with drawn-out tip and mounted at right angles. Air is used at a pressure of 93.0 cm Hg which is kept constant within 2.5 [illegible]. The atomiser consumes about 1.6 ml sample per minute. The burner is [illegible] with a fine mesh and gives a stable flame. Propane gas is used at a pressure of 45 cm H_2O.

For the Na and K analysis a combination of Corning glass filters is used, the characteristics of which are shown in fig. 10.

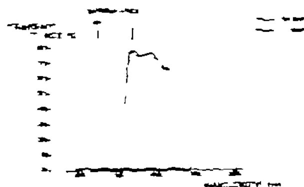


Fig. 10 – Transmittance of filters used in flame photometer

The galvanometers are mounted on a rigid support against the outer wall of the laboratory building. In the course of our studies the Na galvanometer had to be changed (at 10-12-59). The galvanometer scales were such that, with the technique used and in the concentration range observed in our experiments, the smallest difference between two readings corresponded to a difference in Na concentration of respectively 0.25 mEq/l (first galvanometer) and 0.14 mEq/l (second galvanometer). The change of the galvanometer will be neglected in the presentation of our results since we found the same value for the SD of a single plasma Na determination before and after 10-12-60 and no difference in the experimental results was observed (see Addendum). For the K galvanometer the smallest difference between two readings corresponded to 0.0[illegible] mEq/l K.

The following substances were found to have no influence on the emission of an aqueous solution containing 140 mEq/l Na and 5 mEq/l K (all concentrations refer to the undiluted solutions): CaCl (20 mEq/l), MgCl (6 mEq/l), CuSO₄, ZnSO₄, $MnCl_2$, $Fe^{3+}H_2SO_4$, $CdSO_4$, [illegible] (each in a concentration of 0.5 mM/l), the anions SO_4 (135 mEq/l), HCO_3 (140 mEq/l) and PO_4 (100 mM/l) and the organic substances urea (5 g/l) and glucose (10 g/l). In [illegible] found to influence emission since a decontamination of the [illegible] was found to influence emission since a decontamination of the [illegible] of the latter was observed when the pH value of the solution sprayed into the flame was below 6.0. In the dilution used by us the pH was higher than that, unless an acid protein precipitant had been used.

Technique

The initial stages of the procedure differed according to whether plasma (or serum) was analysed directly or after protein precipitation.

When plasma was not deproteinised we proceeded as follows. 0.5 ml plasma was injected with a calibrated syringe-pipet into a 50 ml volumetric flask (Leerdam) and 1 100 diluted with electrolyte-free water

When plasma was deproteinised the following procedure was used. To 0.5 ml plasma 10 ml 6 % trichloroacetic acid was added After centrifugation 8 ml supernatant was pipetted into a 40 ml volumetric flask (Leerdam) and electrolyte-free water added to the mark. In this case the final dilution of plasma was therefore 1 105

In both cases the standard solutions were treated in the same way as the plasma samples.

Four standard solutions were used containing NaCl and KCl in the following concentrations (mEq/L) 120 – 3, 130 – 4 140 – 5 and 150 – 6 With each series of determinations a blank (electrolyte-free water) and at least three of the standard solutions were run. After each sample the standard solution nearest in concentration was re-analysed. If the galvanometer reading for Na had changed, the sample and the standard solution were redetermined until identical readings were obtained for the standard solution before and after the analysis of the sample. Furthermore, when the difference between duplicate determinations was larger than 0.5 mEq/L for Na or 0.05 mEq/L for K, both solutions were re-analysed and the first pair of determinations discarded.

In the case of deproteinised plasma (or serum) the values obtained were always corrected for the error due to the volume change resulting from the removal of protein.

I plasma containing 70 g/L protein with specific volume of 0.8, the protein occupies 5 6 % of the plasma volume. In the procedure for deproteinisation one part of plasma is diluted with 20 parts of 6 % trichloroacetic acid. I the final 1 21 dilution the protein occupies 0.26 of the total volume. As the protein is precipitated, only 99 74 % of the original volume is left, which contains quantitatively the other solutes of the original plasma sample. Consequently their concentration increases with 0 26 %, whereas in the standard solutions, which do not contain protein, no relativ increase in concentration of the solutes occurs. The necessary correction evidently depends on the degree of plasma dilution in the solution from which the protein is precipitated. For 1 6 dilution the correction is — 0.9 %, for 1 2 dilution — 2.8 %.

The values mentioned in the following pages for deproteinised plasma or serum always refer to corrected ones, the correction having been made from the know protein content of the sample and an assumed specific volume of 0 80.

In view of the discussion of systematic errors which is to follow it is *necessary to* mention already here that with the technique described above the SD for a single plasma determination was 0 1 mEq/L for Na and 0.01 mEq/L for K.

Systematic error

The fact that the values published for the normal plasma Na concentration after the introduction of the flame photometer still range from about 135 to about 145 mEq/L and the fact that no common operator can be detected to explain this variation (chapter II) lead one to suspect that systematic errors exist which differ according to the apparatus and technique employed. During the preparation of this manuscript Bergström and Hultman reported that they found both for Na and K a lower value when plasma was analysed than when the solution obtained *after* protein precipitation was analysed. They concluded that the latter value was correct *and that* the low value obtained with plasma was due to a protein interference effect (see chapter II). We also found a difference between Na and K values obtained with plasma (or serum) and those obtained

potassium determination can be done simultaneously No internal lithium standard is used The atomiser consists of two wide bore glass capillaries with drawn-out tip and mounted at right angles Air is used at a pressure of 90 0 cm Hg which is kept constant within 0 5 / The atomiser consumes about 16 ml sample per minute The burner is provided with a fine mesh and gives a stable flame Propane gas is used at a pressure of 45 cm H_2O

For the Na and K analysis a combination of Corning glass filters is used the characteristics of which are shown in fig 10

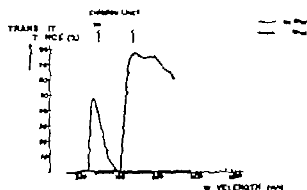


Fig. 10 – Transmittance of filters used in flame photometer

The galvanometers are mounted on a rigid support against the outer wall of the laboratory building. In the course of our studies the Na galvanometer had to be changed (at 10–12–60) The galvanometer scales were such that, with the technique used and in the concentration range observed in our experiments the smallest difference between two readings corresponded to a difference in Na concentration of respectively 0 25 mEq/L (first galvanometer) and 0 14 mEq/L (second galvanometer) The change of the galvanometer will be neglected in the presentation of our results since we found the same value for the SD of a single plasma Na determination before and after 10–12–60, and no difference in the experimental results was observed (see Addendum) For the K galvanometer the smallest difference between two readings corresponded to 0 02 mEq/L K.

The following substances were found to have no influence on the emission of an aqueous solution containing 140 mEq/L Na and 5 mEq/L K (all concentrations refer to the undiluted solutions) $CaCl_2$ (20 mEq/L) $MgCl_2$ (8 mEq/L) $CuSO_4$, $ZnSO_4$, $MnCl_2$ $Fe(NH_4)_2SO_4$, $CdSO_4$, ammoniummolybdate (each in a concentration of 0 5 mM/L) the anions SO_4 (145 mEq/L), HCO_3 (140 mEq/L) and PO_4 (100 mM/L) and the organic substances urea (6 g/L) and glucose (10 g/L) In confirmation of the litterature H-ions were found to influence emission since a depression of the latter was observed when the pH value of the solution sprayed into the flame was below 6.0 In the dilution used by us the pH was higher than that, unless an acid protein precipitant had been used

Technique

The initial stages of the procedure differed according to whether plasma (or serum) was analysed directly or after protein precipitation

When plasma was not deproteinised we proceeded as follows. 0.5 ml plasma was injected with a calibrated syringe-pipet into a 50 ml volumetric flask (Leerdam) and 1 : 100 diluted with electrolyte-free water.

When plasma was deproteinised the following procedure was used. To 0.5 ml plasma 10 ml 6 % trichloroacetic acid was added. After centrifugation 8 ml supernatant was pipetted into a 40 ml volumetric flask (Leerdam) and electrolyte-free water added to the mark. In this case the final dilution of plasma was therefore 1 : 105.

In both cases the standard solutions were treated in the same way as the plasma samples.

Four standard solutions were used containing NaCl and KCl in the following concentrations (mEq/L): 120 – 3, 130 – 4, 140 – 5 and 150 – 6. With each series of determinations a blank (electrolyte-free water) and at least three of the standard solutions were run. After each sample the standard solution nearest in concentration was re-analysed. If the galvanometer reading for Na had changed, the sample and the standard solution were redetermined until identical readings were obtained for the standard solution before and after the analysis of the sample. Furthermore, when the difference between duplicate determinations was larger than 0.5 mEq/L for Na or 0.05 mEq/L for K, both solutions were re-analysed and the first pair of determinations discarded.

In the case of deproteinised plasma (or serum) the values obtained were always corrected for the error due to the volume change resulting from the removal of protein.

In plasma containing 70 g/L protein with a specific volume of 0.8, the protein occupies 5.6 % of the plasma volume. In the procedure for deproteinisation one part of plasma is diluted with 20 parts of 6 % trichloroacetic acid. In the final 1 : 21 dilution the protein occupies 0.26 % of the total volume. As the protein is precipitated, only 99.74 % of the original volume is left, which contains quantitatively the other solutes of the original plasma sample. Consequently their concentration increases with 0.26 %, whereas in the standard solutions, which do not contain protein, no relative increase in concentration of the solutes occurs. The necessary correction evidently depends on the degree of plasma dilution in the solution from which the protein is precipitated. For a 1 : 6 dilution the correction is – 0.9 %, for a 1 : 2 dilution – 2.8 %.

The values mentioned in the following pages for deproteinised plasma or serum always refer to corrected ones, the correction having been made from the known protein content of the sample and an assumed specific volume of 0.80.

In view of the discussion of systematic errors which is to follow, it is necessary to mention already here that with the technique described above the SD for a single plasma determination was 0.1 mEq/L for Na and 0.01 mEq/L for K.

Systematic error

The fact that the values published for the normal plasma Na concentration after the introduction of the flame photometer still range from about 135 to about 145 mEq/L and the fact that no common operator can be detected to explain this variation (chapter II) lead one to suspect that systematic errors exist which differ according to the apparatus and technique employed. During the preparation of this manuscript Bergström and Hultman reported that they found, both for Na and K, a lower value when plasma was analysed than when the solution obtained after protein precipitation was analysed. They concluded that the latter value was correct and that the low value obtained with plasma was due to a protein interference effect (see chapter II). We also found a difference between Na and K values obtained with plasma (or serum) and those obtained

with deproteinised plasma (or serum) but from evidence to be presented below we must conclude that *with our technique and apparatus* for Na the values obtained with plasma or serum are nearly correct (0.1 mEq/L *too low*) *whereas the values obtained after* deproteinisation are on the average 2.5 mEq/L too high. For K these values are respectively 0.1 mEq/L too low and 0.1 mEq/L too high. To add a new term to the one coined by Bergström and Hultman *with our technique the protein interference effect is very small but the deproteinisation effect fairly large in the case of Na whereas they are of about equal magnitude in the case of K*

Apparently the protein interference effect and the deproteinisation effect vary for each apparatus and technique At present we still have no satisfactory explanation for either of the two effects *These are being further studied in our department by* De Vries and Van Daatselaar and here we will only present the data from which we calculated the magnitude of these effects in our experiments

Magnitude of the difference found between plasma (or serum) and deproteinised plasma (or serum) when using our technique In 1958 De Vries and Van Daatselaar observed that both the Na and the K concentration were found 1–3 % lower when plasma (or serum) was analysed directly in the flame photometer than when it was analysed after deproteinisation (protein precipitated with 6 % trichloroacetic acid 1 : 21 concentration corrected for volume change due to removal of protein) [unpublished results]

In our experiments we consistently found a difference between plasma and deproteinised plasma both for normal and for pathological plasma (or serum), including that obtained from patients with myeloma and severe uraemia. The difference was the same when protein precipitants other than trichloroacetic acid were used (HCl perchloric acid, acetic acid, uranylacetate) provided protein precipitation occurred at the same plasma dilution (*i.e.* 1 : 21)

The magnitude of the difference is demonstrated by the results obtained in the 19 *in vivo* ultrafiltration experiments in which Na and K were determined both in plasma and in deproteinised plasma (table 14) For Na there appears to be a positive correlation with the protein concentration of the plasma

Evidence that with our technique the values obtained with plasma are very nearly correct This evidence is mainly provided by experiments in which Na and K concentration were analysed in plasma before and after ashing, and by experiments in which electrolyte-free serum was added in increasing amounts to a fixed quantity of a standard solution containing NaCl and KCl. The series of ashing experiments to be reported did not include the ashing of deproteinised plasma, since at the time the ashing of the protein free supernatant failed to give reproducible results presumably because of the large quantity of fluid which has to be ashed This increases the chances that in the course of the ashing procedure losses occur as a result of splattering

(1) Ashing experiments – Fresh plasma samples with a protein concentration ranging from 50 to 160 g/L were analysed for Na and K, in a number of cases also the deproteinised plasma (6 % trichloroacetic acid, plasma dilution 1 : 21) The plasma samples were then ashed, together with samples of standard solution and the Na and K content of the ash determined.

Silica crucibles containing 0.5 ml sample + 0.5 ml 4 N H_2SO_4 were heated for at least 2 hours on a steam bath, dried in an electric sterilizer at 110°C for one night and finally transferred to an electric furnace the temperature of which was gradually raised from 200°C to 500°C. As soon as their contents had become completely white (after 5 to 8 days), the crucibles were removed, the ash quantitatively dissolved in distilled water to a total volume of 50 ml, and the solution analysed for Na and K. As the standard solutions always gave the same galvanometer reading before and after the ashing, it would appear that no Na or K was lost. When in later experiments it was found that no loss of Na and K occurred at 700°C, the period in the furnace could be shortened to 4 hours at 700°C. The ash was found to contain sulphate, very small amount of phosphate, no chloride and no ammonium.

The results are presented in table 15 It can be seen that in the case of Na the concentration found in plasma is on the average 0 1 mEq/L lower than that found in the ash. The difference ranges from +0.8 to −1.0 mEq/L, and no correlation was found with the protein concentration of the sample. In the 12 instances in which deproteinised plasma (not ashed!) has also been analysed, the latter values were on the average 2.3 mEq/L higher than the values obtained on plasma.

In the case of K the concentration in plasma is on the average 0.11 mEq/L lower than in the ash, the difference ranging from +0.08 to −0.32. This covers about ⅓ of the difference observed between plasma and deproteinised plasma but – as in the case of Na there appears to be no correlation with protein concentration.

(2) ADDITION OF ELECTROLYTE-FREE SERUM TO STANDARD SOLUTION – Serum was dialysed, for 24 hours at 20°C under sterile conditions in Visking cellophan bags, against distilled water The *pH* of the latter was kept at about 5.0 by the addition of HCl After dialysis the serum samples contained less than 0.5 mEq/L (Na + K). Increasing amounts of the serum were then added to a fixed amount of standard solution and the effect on the flame photometer reading was determined.

Into 50 ml volumetric flasks 0.5 ml of standard solution, containing 140 mEq/L NaCl and 5 mEq/L KCl, was injected and respectively 0 0.5, 1.0 1.5 etc. ml of the dialysed serum added. One flask always contained 4 ml serum without NaCl solution in order to determine the amount of Na and K still present in the dialysed serum. The flasks were then filled to the 50 ml mark with distilled water and the contents analysed in the flame photometer, together with set of standard solutions The galvanometer readings obtained were expressed in terms of mEq/L, corrected for the amount of Na or K still present in the serum after dialysis, and then plotted against the protein concentration of the flasks content, multiplied by 100. In this way protein concentration is expressed as if plasma samples with increasing protein concentration had been analysed in dilution of 1 100

From table 16 and fig 11 it can be seen that protein concentrations higher than 30 g/L have an increasingly depressive effect on the flame photometer reading, but in the range generally observed in the *in vitro* ultrafiltration experiments, *i.e.* 60 to 120 g/L, the magnitude of the depression remains small in the case of Na. In the plasma of patients with myeloma occasionally protein concentrations as high as 160 g/L were observed and in that case a more severe depression might be expected to occur The effect on K determination has been studied in one experiment only as in the ashing experiments it is evident that the percentage error is considerably larger than in the case of Na, but the same trend exists.

with deproteinised plasma (or serum) but from evidence to be presented below we must conclude that *with our technique and apparatus* for Na the values obtained with plasma or serum are nearly correct (0 1 mEq/L too low) whereas the values obtained after deproteinisation are on the average 2.5 mEq/L too high For K these values are respectively 0 1 mEq/L too low and 0 1 mEq/L too high. To add a new term to the one coined by Bergström and Hultman *with our technique the protein interference effect is very small but the deproteinisation effect fairly large in the case of Na whereas they are of about equal magnitude in the case of K*

Apparently the protein interference effect and the deproteinisation effect vary for each apparatus and technique *At present we still have no satisfactory explanation for* either of the two effects These are being further studied in our department by DE VRIES and VAN DAATSELAAR and here we will only present the data from which we calculated the magnitude of these effects in our experiments

Magnitude of the difference found between plasma (or serum) and deproteinised plasma (or serum) when using our technique In 1958 DE VRIES and VAN DAATSELAAR observed that both the Na and the K concentration were found 1–3 / lower when plasma (or serum) was analysed directly in the flame photometer than when it was analysed after deproteinisation (protein precipitated with 6 / trichloroacetic acid *1 21* concentration corrected for volume change due to removal of protein) [unpublished results].

In our experiments we consistently found a difference b-tween plasma and deproteinised plasma both for normal and for pathological plasma (or serum) including that obtained from patients with myeloma and severe uraemia. Th difference was the same when protein precipitants other than trichloroacetic acid were used (HCl perchloric acid, acetic acid uranylacetate) provided protein precipitation occurred at the same plasma dilution (*i e* 1 21)

The magnitude of the difference is demonstrated by the results obtained in the 19 *in vivo* ultrafiltration exp-riments in which Na and K were determined both in plasma and in deproteinised plasma (table 14) For Na there appears to be a positive correlation with the protein concentration of the plasma

Evidence that with our technique the values obtained with plasma are very nearly correct This evidence is mainly provided by experiments in which Na and K concentration were analysed in plasma before and after ashing, and by experiments in which electrolyte free serum was added in increasing amounts to a fixed quantity of a standard solution containing NaCl and KCl. The series of ashing experiments to be reported did not include the ashing of deproteinised plasma, since at the time the ashing of the protein-free supernatant failed to give reproducible results, presumably because of the large quantity of fluid which has to be ashed This increases the chances that in the course of the ashing procedure losses occur as a result of splattering

(1) ASHING XPERIMENTS – Fresh plasma samples with a protein concentration ranging from 50 to ‿0 g/L were analysed for Na and K, in a number of cases also the deproteinised plasma (6 / trichloroacetic acid *plasma dilution 1 21)* The plasma samples were then ashed together with samples of standard solution and the Na and K content of the ash determined.

deproteinisation effect of the order of 2.5 mEq/L in the case of Na (provided the protein is precipitated by 6 / trichloroacetic acid 1 21).

Systematic error of Na and K determination in plasma (or serum) in our experiments. In the case of Na the ashing experiments indicate that the average error is 0.1 mEq/L, whereas from the results of the experiments in which electrolyte-free serum was added to standard solution the average error would appear to be 0.2 mEq/L at 70 g/L protein and 0.4 mEq/L at 120 g/L protein. This effect of an increase in protein concentration was not observed in the ashing experiments, where the accuracy is necessarily somewhat less. Table 16 moreover demonstrates that different plasma samples vary greatly in this respect. In view of the fact that considerably more samples were studied in the ashing experiments, the following conclusions appear reasonable.

On the average, Na concentration is underestimated by 0.1 mEq/L at a protein concentration of 60 g/L, and this error doubles when the protein concentration increases from 60 to 120 g/L. In the case of K the percentage error is considerably larger and from the experimental evidence presented we conclude that here also the underestimate is on the a erage 0.1 mEq/L at a protein concentration of 60 g/L, and doubles when the protein concentration increases from 60 to 120 g/L.

No attempt will be made to correct individual values for this error but it will be considered in the discussion of our results.

Ac idental error

As mentioned before, the standard solution was redetermined after each sample, and the value obtained for the latter was only accepted if the standard readings before and after were identical. The gain in reproducibility is evident from table 17 which compares the results obtained by measuring 10 d lutions (1 100) of the same plasma in the manner described (A), with those obtained by simply determining the standard solution between samples and calculating the sample alue by interpolation (B). The degree of reproducibility in series A (table 17) is of the same order as the SD of a single determination and the SE of the mean of duplicate determinations calculated from the 543 duplicate determinations obtained in the *in vivo* ultrafiltration experiments (table 18).

Since normally the duplicate determination is known as such, bias could have influenced the reading of the duplicate, and the results of the duplicate determinations would then erroneously suggest a high reproducibility The following experiment was therefore done From two standard solutions, containing respectively 130.0 mEq/L NaCl + 4 0 mEq/L KCl and 140 0 mEq/L NaCl + 5 0 mEq/L KCl, the following mixtures were prepared w th calibrated pipets 10 + 0 ml, 9 + 1 ml, 8 + 2 ml, 0 + 10 ml. From the resulti ng solutions 1 100 dilutions were prepared in duplicate by the person who did the flame photometer determinations. Someone else then put the flasks in a random order unknown to the first person. After completing the determinations the values obtained were compared w th the calculated values. Fig. 13 demonstrates that under these circumstances the reproducibility remained of the same order as that calculated from the duplicate determinations in the *in vivo* ultrafiltration experiments. For Na the d fference between duplicate alues was not more than 0.4 mEq/L and for K not more than 0.09 mEq/L. The mean values are close to the calculated values, with one notable xception. For the solution with a calculated concentration of 131.0 + 4 1 a mean

The results of the ashing experiments and those obtained by adding serum to standard solution are in reasonable agreement for the range 50–120 g/L protein It would appear that in normal plasma the depressive effect of protein on Na emission is almost negligible and not larger than in dialysed serum. However the relatively small difference between plasma and ashed plasma obtained in 4 hyperproteinaemic (and hypalbuminaemic) samples of the two patients with myeloma (Fr and Ja in table 15) suggests that

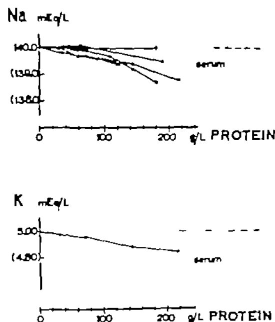


Fig. 11 Effect of adding increasing amounts of electrolyte-free serum to a NaCl and KCl containing standard solution (see table 15).

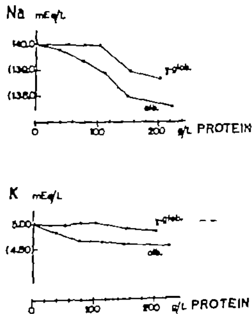


Fig. 12 – Effect of adding increasing amounts of electrolyte-free albumin, respectively γ-globulin, to a NaCl and KCl containing standard solution.

the proteins differ in their depressive effect. We have therefore also done some experiments in which either albumin or γ globulin was added to a standard solution in the manner described above The results are shown in fig 12. They indicate that the depression is more pronounced in the case of albumin than in the case of γ-globulin

The effect of increasing protein concentration on the emission presumably is the result of a change in viscosity or surface tension It is to be expected that the protein concentration at which the depressive effect becomes apparent will be different for different apparatus and will depend on the characteristics of the atomiser But this explanation is not completely satisfactory since the effect is relatively larger for K than for Na

After this chapter was written Van Daatselaar finally succeeded in ashing deproteinised plasma (6 / trichloroacetic acid 1 21) with the same accuracy as plasma. In preliminary experiments again practically no difference was observed between the Na concentration of plasma before and after ashing and again the Na concentration of the deproteinised plasma was found to be 2–4 mEq/L higher than that of plasma. But in addition it was now found that the Na concentration of the ashed deproteinised plasma was equal to the concentration found in plasma to within 0.1 mEq/L.

The latter experiments confirm our conclusion that when plasma (or serum) is analysed with our technique, the protein interference effect is of the orde

deproteinisation effect of the order of 2.5 mEq/L in the case of Na (provided the protein is precipitated by 6 / trichloroacetic acid 1 21).

Systematic error of Na and K determination in plasma (or serum) in our experiments. In the case of Na the ashing experiments indicate that the average error is 0 1 mEq/L, whereas from the results of the experiments in which electrolyte-free serum was added to standard solution, the average error would appear to be 0.2 mEq/L at 70 g/L protein and 0 4 mEq/L at 120 g/L protein. This effect of an increase in protein concentration was not observed in the ashing experiments, where the accuracy is necessarily somewhat less Table 16 moreover demonstrates that different plasma samples vary greatly in this respect. In view of the fact that considerably more samples were studied in the ashing experiments, the following conclusions appear reasonable.

On the average Na concentration is underestimated by 0.1 mEq/L at a protein concentration of 60 g/L, and this error doubles when the protein concentration increases from 60 to 120 g/L. In the case of K the percentage error is considerably larger and from the experimental evidence presented we conclude that here also the underestimate is on the average 0.1 mEq/L at a protein concentration of 60 g/L, and doubles when the protein concentration increases from 60 to 120 g/L.

No attempt will be made to correct individual values for this error but it will be considered in the discussion of our results.

Accidental error

As mentioned before, the standard solution was redetermined after each sample, and the alue obtained for the latter was only accepted if the standard readings before and after were identical. The gain in reproducibility is evident from table 17 which compares the results obtained by measuring 10 dilutions (1 100) of the same plasma in the manner described (A), with those obtained by simply determining the standard solution between samples and calculating the sample value by interpolation (B). The degree of reproducibility n series A (table 17) is of the same order as the SD of a single determination and the SE of the mean of duplicate determinations calculated from the 543 duplicate determinations obtained in the *in vitro* ultrafiltration experiments (table 18).

Since normally the duplicate determination is known as such, bias could have influenced the reading of the duplicate, and the results of the duplicate determinations would then erroneously suggest a high reproducibility The following experiment was therefore done From two standard solutions, containing respectively 130.0 mEq/L NaCl + 4 0 mEq L KCl and 140 0 mEq/L NaCl + 5 0 mEq/L KCl, the following mixtures were prepared w th calibrated pipets 10 + 0 ml, 9 + 1 ml, 8 + 2 ml, 0 + 10 ml. From the resulting solutions 1 100 dilutions were prepared in duplicate by the person who did the flame photometer determinations. Someone else then put the flasks in a random order unknown to the first person. After completing the determinations the values obtained were compared with the calculated values. Fig. 13 demonstrates that under these circumstances the reproducibility remained of the same order as that calculated from the duplicate determinations in the *in vitro* ultrafiltration experiments. For Na the d fference between duplicate alues was not more than 0.4 mEq/L and for K not more than 0 09 mEq/L The mean values are close to the calculated values, with one notable exception. For the solution with a calculated concentration of 131 0 + 4 1 a mean

value of 132.0 + 4.2 was found, the duplicate values showing practically no difference. This value was consistently found on repeated determinations. It is therefore possible that the discrepancy is due to an error in the preparation of the solutions. This stresses the fact that in fig. 13 the results of the 'blind' analysis are presented somewhat unfavourably because the error involved in making up the solutions was assumed to be zero whereas even with calibrated pipets an error of 0.1 % cannot be avoided.

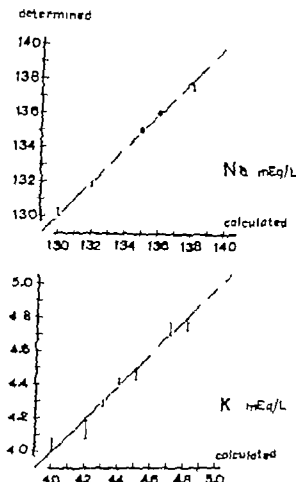


Fig. 13. Values calculated compared with values determined in 'blind' analysis of duplicate samples of mixtures from two different NaCl + KCl solutions.

It is therefore concluded that the influence of bias on the results of Na and K analysis can be neglected.

Ca and Mg

These were determined by photoelectric colorimetric titration with EDTA (Gorter & De Graaff 1955).

Technique

We used a micrometer buret, and magnetic stirring. The transmittance was read on a galvanometer to ½ scale unit. Titration proceeded as follows:

(a) *Calcium*. With a calibrated syringe-pipet 0.5 ml sample is injected into 6 ml 0.05 N NaOH with murexide as indicator. Then titration with 1.5 EDTA is started

transmittance being read at 565 mμ. Near the equivalence point EDTA is added in small amounts and the resulting transmittances are noted. This is continued until transmittance remains constant (the concentrations of the reagents are so chosen that the transmittance is 35–55 % at this point). Finally the amounts of EDTA successively added are plotted against the concomitant transmittances, and the equivalence point is determined by graphic interpolation.

(b) *Calcium + magnesium*. If the sumtitration immediately follows Ca titration, the pH of the titration milieu is first lowered by adding 0.1 ml 3 N NH_4Cl. Then eriochrom black is added as indicator and the wavelength changed to 660 mμ. If only a sumtitration is done 0.5 ml sample is added to 6 ml 0.05 N NH_4OH with eriochrom black. In both cases the further procedure is as described above.

With each series of analyses a reagent blank and a standard solution (5.0 mEq/L $CaCl_2$ + 2.0 mEq/L $MgCl_2$) are determined. Mg is obtained by subtraction. This is permitted since the same quantity of EDTA is needed to titrate 1 mEq of Ca and 1 mEq of Mg respectively.

Systematic error

When the sumtitration follows directly on Ca titration an error may be made. It was found that in the strongly alkaline milieu used for the Ca titration Mg is precipitated from a standard solution, but not from a protein containing solution. After the pH has been lowered by adding 3 N NH_4Cl, the finely dispersed precipitate redissolves in the course of 4 minutes. The sumtitration will therefore give erroneously low values for the standard solution if it is started immediately after adding the NH_4Cl. Since protein prevents the precipitation of Mg, no error occurs with plasma, and a 3–4 % overestimate of plasma Mg concentration is the result. However, when sufficient time is allowed for Mg to redissolve, the titration of Ca + Mg following Ca titration gives the same value as the direct sumtitration.

Initially a solution containing 1.0 mEq/L $CaCl_2$ + 1.0 mEq/L $MgCl_2$ was added to one of the plasma samples at the end of each series of determinations. When it was found that recovery was always between 97 and 103 % of the calculated amount, this practice was abandoned. No difference was found between plasma and deproteinised plasma (6 % trichloroacetic acid 1 : 21) and even in haemolytic plasma no disturbing influence of Fe could be detected.

Accidental error

From the *in vivo* ultrafiltration experiments the SD of a single determination and the SE of the mean of a duplicate determination were calculated, both for Ca (509 duplicate determinations) and for the sumtitration of Ca and Mg (564 duplicate determinations) (table 19). For both determinations the absolute value of the SD was the same at high and at low concentrations.

Cl

This was determined by potentiometric titration with silvernitrate. The method is essentially that described by SANDERSON (1952), but a number of modifications have been made by DE VRIES.

Apparatus

Instead of a buret a 2 ml glass syringe driven by a micrometer is used. The latter is graduated to 0 01 mm and has a length of 25 mm. To the tip of the syringe a plastic cannula is fitted with a silver wire projecting into its lumen (reference electrode). The measuring electrode is another silver wire which, with the tip of the cannula, reaches down into the flatbottom titration vessel (2 × 5 cm) The two silver wires are connected to a direct reading electronic millivoltmeter A magnetic stirrer is used.

Technique

After filling syringe and cannula with 0 5 N $AgNO_3$ (care being taken that no air bubbles are trapped) the measuring electrode is rubbed with sandpaper and cleaned with distilled water Then the titration vessel is filled with about 7 ml 0.2 N H_2SO_4 in 0.2 % chloride free gelatin (the gelatin was added to prevent precipitation of AgCl on the measuring electrode) Finally 0 5 ml sample is added from a calibrated syringe-pipet. After lowering the measuring electrode and cannula tip into the titration vessel, the voltmeter is adjusted for sensitivity and zero and the titration is started. When nearing the equivalence point, $AgNO_3$ is added in very small amounts and sufficient time is allowed for the meter to adjust itself In accordance with the experience of Sanderson it was found that the end point is very constant and occurs at the same potential in standard solutions and in plasma. Although for the latter the slope of the titration curve is slightly less steep it is our experience that the end point can be read without difficulty For that reason we no longer make a graph of every titration, but titrate to the known potential of the equivalence point. With every series of analyses titrations were done on the H_2SO_4-gelatin solution (serving as a blank) and on two of the three available standard solutions which contained 100 0 123 0 and 134 0 mEq/L of Cl respectively The latter two were the 120 0 + 3 0 and 130 0 + 4 0 standard solutions used with the Na and K determinations During a series of determinations one of the standard solutions was measured repeatedly in order to detect alterations in the electrode sensitivity When the difference between duplicates was more than 1 0 mEq/L new duplicate determinations were done and the first pair was discarded

Systematic error

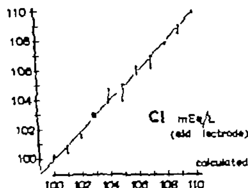
The presence of protein had no effect on the titration results. The addition of increasing amounts of electrolyte free plasma to 0 5 ml NaCl standard solution had no influence on the Cl concentration measured (table 20) and the Cl concentration measured in deproteinised plasma (6 % trichloroacetic acid 1 11) did not differ significantly from that measured in plasma (table 21) Furthermore the use of the same standard solutions for Na and K analysis and for Cl analysis excludes one source of systematic error in the calculation of the difference between cation and anion concentrations.

In this connection the following experiment is of interest Three polyethylene containers (used in the dialysis experiments to be mentioned in chapter V) and one glass container were filled with a NaCl solution and left to stand at 38°C for 40 hours. During this period several samples were taken from each container and analysed for Na and for Cl, the latter both with the potentiometric titration and with the method of Volhard as described in GORTER & DE GRAAFF (1955). The experiment was devised to see whether during dialysis experiments Na or Cl were released from, or absorbed by the wall of the containers. The results of the experiments are shown in table 22 It can be seen that the Na determination and the potentiometric Cl determination giv nearly

identical results and that their reproducibility is of the same magnitude. In contrast, the Cl values obtained with the Volhard method are less reproducible and on the average significantly lower than the Na concentrations, although the same standard solutions were used for all three methods.

Accidental error

The SD of a single determination, calculated from the 556 duplicate determinations done in the *in vivo* ultrafiltration experiments, was 0.11 mEq/L, and the SE of the mean 0.08 mEq/L (Cl concentrations ranging from 95 to 106 mEq/L plasma).



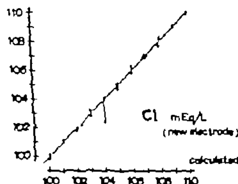


Fig. 14 Values calculated compared with values determined in "blind" analysis of duplicate samples of mixtures from two different NaCl solutions

Solutions with increasing Cl concentrations were prepared from two standard solutions, containing respectively 100.0 mEq/L and 110 mEq/L NaCl. The samples obtained were analyzed "blind" after the manner described for Na and K. Fig. 14 represents the result of the two experiments done. In one the measuring electrode was rather aged in the other experiment it was new. With the new electrode the standard deviation of a single determination did not differ significantly from that calculated above. The only value which differed more than 0.3 mEq from the calculated value was indeed due to an error in determination on redetermination a value of 104.5 instead of 102.4 was found. In the experiment with the old electrode the standard deviation was about

Apparatus

Instead of a buret a 2 ml glass syringe driven by a micrometer is used. The latter is graduated to 0.01 mm and has a length of 25 mm. To the tip of the syringe a plastic cannula is fitted with a silver wire projecting into its lumen (reference electrode). The measuring electrode is another silver wire which, with the tip of the cannula, reaches down into the flatbottom titration vessel (2 × 5 cm). The two silver wires are connected to a direct reading electronic millivoltmeter. A magnetic stirrer is used.

Technique

After filling syringe and cannula with 0.5 N $AgNO_3$ (care being taken that no air bubbles are trapped) the measuring electrode is rubbed with sandpaper and cleaned with distilled water. Then the titration vessel is filled with about 7 ml 0.2 N H_2SO_4 in 0.2 chloride-free gelatin (the gelatin was added to prevent precipitation of AgCl on the measuring electrode). Finally 0.5 ml sample is added from a calibrated syringe-pipet. After lowering the measuring electrode and cannula tip into the titration vessel, the voltmeter is adjusted for sensitivity and zero and the titration is started. When nearing the equivalence point, $AgNO_3$ is added in very small amounts and sufficient time is allowed for the meter to adjust itself. In accordance with the experience of Sanderson it was found that the end point is very constant and occurs at the same potential in standard solutions and in plasma. Although for the latter the slope of the titration curve is slightly less steep it is our experience that the end point can be read without difficulty. For that reason we no longer make a graph of every titration but titrate to the known potential of the equivalence point. With every series of analyses titrations were done on the H_2SO_4-gelatin solution (serving as a blank) and on two of the three available standard solutions which contained 100.0, 123.0 and 134.0 mEq/L of Cl respectively. The latter two were the 120.0 + 3.0 and 130.0 + 4.0 standard solutions used with the Na and K determinations. During a series of determinations one of the standard solutions was measured repeatedly in order to detect alterations in the electrode sensitivity. When the difference between duplicates was more than 1.0 mEq/L new duplicate determinations were done and the first pair was discarded.

Systematic error

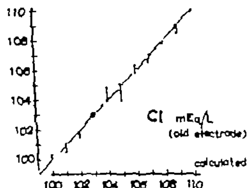
The presence of protein had no effect on the titration results. The addition of increasing amounts of electrolyte free plasma to 0.5 ml NaCl standard solution had no influence on the Cl concentration measured (table 20) and the Cl concentration measured in deproteinised plasma (6 % trichloroacetic acid 1 : 11) did not differ significantly from that measured in plasma (table 21). Furthermore the use of the same standard solutions for Na and K analysis and for Cl analysis excludes one source of systematic error in the calculation of the difference between cation and anion concentrations.

In this connection the following experiment is of interest. Three polyethylene containers (used in the dialysis experiments to be mentioned in chapter V) and one glass container were filled with a NaCl solution and left to stand at 38°C for 40 hours. During this period several samples were taken from each container and analysed for Na and for Cl, the latter both with the potentiometric titration and with the method of Volhard as described in GORTER & DE GRAAFF (1955). The experiment was devised to see whether during dialysis experiments Na or Cl were released from, or absorbed by the wall of the containers. The results of the experiments are shown in table 22. It can be seen that the Na determination and the potentiometric Cl determination give nearly

identical results and that their reproducibility is of the same magnitude. In contrast, the Cl values obtained with the Volhard method are less reproducible and on the average significantly lower than the Na concentrations, although the same standard solutions were used for all three methods.

Accidental error

The SD of a single determination, calculated from the 556 duplicate determinations done in the *in vivo* ultrafiltration experiments, was 0.11 mEq/L, and the SE of the mean 0.08 mEq/L (Cl concentrations ranging from 95 to 106 mEq/L plasma).



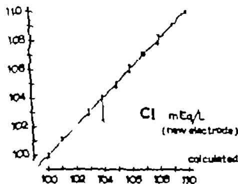


Fig. 14. Values calculated compared with values determined in "blind" analysis of duplicate samples of mixtures from two different NaCl solutions.

Solutions with increasing Cl concentrations were prepared from two standard solutions containing respectively 100.0 mEq/L and 110 mEq/L NaCl. The samples obtained were analysed 'blind' after the manner described for Na and K. Fig. 14 represents the results of the two experiments done. In one the measuring electrode was rather aged, in the other experiment it was new. With the new electrode the standard deviation of a single determination did not differ significantly from that calculated above. The only value which differed more than 0.3 mEq from the calculated value was indeed due to an error in determination: on redetermination a value of 104.5 instead of 102.4 was found. In the experiment with the old electrode the standard deviation was about

four times as large (SD 0 40 mEq/L) but on the average the difference between the calculated and the measured concentration was less than 0 1 mEq/L.

Although there is therefore no evidence of bias, the difference in the results obtained with the two electrodes indicates that the reproducibility of the Cl determination must have varied *in the one and a half year during which our experiments were done. However* the policy of redetermining duplicate samples when they differed by 1 mEq/L or more,

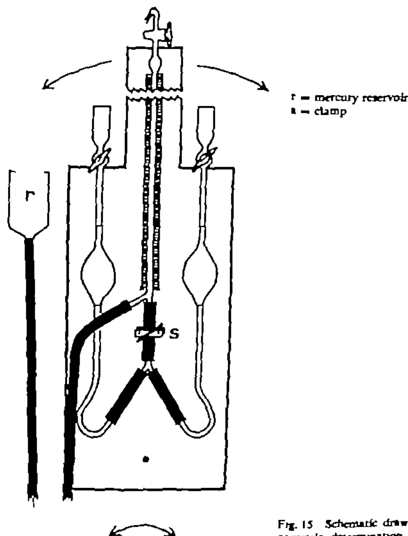


Fig. 15 Schematic drawing of apparatus used for manometric determination of CO content

should have had a stabilising influence For that reason and *because all our measurements* were done with a new or fairly new electrode we accept the SE of the mean of a duplicate determination *as calculated from* the *in vivo* ultrafiltration experiments (*i e* 0 08 mEq/L).

Total CO_2
This was determined by the manometric method of VAN SLYKE & NEILL (1924)

Apparatus

We used the apparatus devised by Borst for gasometric urea determination. Its essentials are described by Bots (1942) and a schematic presentation is given in fig. 15

It consists of 2 extraction chambers with manometer tube in between. The glass parts are connected by thick-walled rubber tubing and the whole is mounted on a vertical wooden board and rigidly fixed. The board is made to shake in vertical plane by means of motor with excentric transmission. The lower end of the extraction chamber units has the shape of an inverted question mark, so that air leaking through the connection with the manometer tube enters the latter and can be expelled before reading the gas pressure, by temporarily closing screw and raising the mercury reservoir. The manometer tube thus serves as an air trap, which arrangement limits the total number of stopcocks to three. Before reading gas pressure, the manometer is placed exactly vertical with the aid of levelling device mounted on the board.

The advantages of the apparatus are that duplicate determinations can be done simultaneously and that errors due to leakage are largely prevented.

Technique

0.2 ml samples are used and injected with a calibrated syringe-pipet. With our apparatus one minute shaking at 200 revolutions/min. was found amply sufficient for complete extraction. The height of the mercury column is read to the nearest 0.5 mm. Otherwise technique, reagents, storage of reagents and correction factors are those described by Van Slyke and Neill (see also PETERS & VAN SLYKE 1932)

With each series of analyses a standard solution containing 25 mM/L Na_2CO_3 is determined. It is prepared by dissolving 1.325 g dry Na_2CO_3 (analytical grade) in distilled CO_2-free water to a total volume of 500 ml, and the concentration checked by titration with 0.1000 N HCl standard solution. The carbonate solution is stored in a closed pyrex bottle and a fresh solution is made every fortnight.

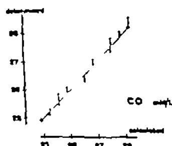


Fig. 14 Values calculated compared with values determined in "blind" analysis of duplicate samples of mixtures from two different Na_2CO_3 solutions.

Systematic error

For this the reader is referred to PETERS & VAN SLYKE (193). It would appear that no systematic error arises if sufficient attention is given to the details of the technique as described by Van Slyke and Neill.

Accidental error

From the 617 duplicate analyses performed in the in vivo ultrafiltration experiments the SD of single determination was calculated at 0.20 mM/L, and the SE of the mean

four times as large (SD 0 40 mEq/L) but on the average the difference between the calculated and the measured concentration was less than 0 1 mEq/L.

Although there is therefore no evidence of bias the difference in the results obtained with the two electrodes indicates that the reproducibility of the Cl determination must have varied in the one and a half year during which our experiments were done. However the policy of redetermining duplicate samples when they differed by 1 mEq/L or more,

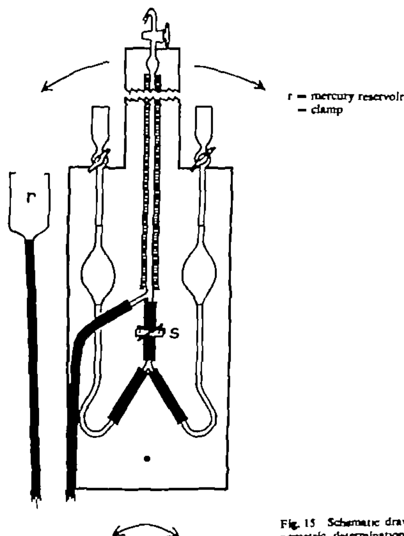


Fig. 15 Schematic drawing of pparatus used for manometric determination of CO content

should have had a stabilising influence For that reason and because all our measurements were done with a *new* or fairly new electrode, we accept the SE of the mean of a duplicate determination as calculated from the *in vivo* ultrafiltration experiments (*i.e.* 0 08 mEq/L)

Total CO_2
This was determined by the manometric method of VAN SLYKE & NEILL (1924)

The buffers were stored in closed pyrex glass containers at 20°C. Just prior to a series of determinations a small amount was decanted in stoppered glass tubes to be used for that day only. Fresh buffer solutions were prepared every two weeks. The difference between the 7.100 and the 7.400 buffer solutions was always 0.300 ± 0.003 pH units.

Technique

After flushing the glass electrode several times with the sample fluid, equilibrium is generally reached within half a minute. The potential was read for four consecutive minutes and the difference between the last two readings was not more than 0.003 pH units unless the glass electrode had become too old. After each measurement the electrode was flushed with a 0.15 molar NaCl solution. At the conclusion of a series of determinations the electrode was stored with 7.400 buffer solution. A series of determinations was always started by measuring both buffer solutions repeatedly and alternatingly; the buffer nearest to the pH of the samples was remeasured after every 2 to 3 samples.

All determinations were done in duplicate, and in our experiments the difference did not exceed 0.005 pH unit. In the *in vivo* ultrafiltration and the dialysis experiments the pH values were rounded off to the nearest 0.01 pH unit. This was thought to be sufficiently accurate for the purpose of the present investigation.

Finally, to obtain the pH values for "true" plasma, 0.01 was added to the blood pH values as a correction for the suspension effect of erythrocytes on the glass electrode (SEVERINGHAUS *et al.* 1956[1]). The pH values so obtained were used in the calculation of plasma bicarbonate.

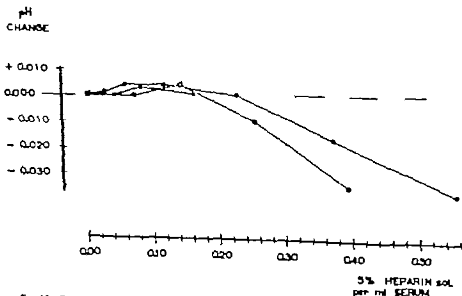


Fig. 17. Determination of pH. Effect of adding undiluted 5 % heparin solution to serum (3 samples)

at 0 14 mM/L. As with Na K and Cl the influence of bias on the duplicate results was tested by blind determination of a series of duplicate mixtures of a 24.9 mM/L and a 28 2 mM/L Na_2CO_3 standard solution The results are shown in fig. 16 and indicate that bias is not likely to have had a significant influence on our results.

pH

Handling and storing of samples

In the *in vivo* ultrafiltration experiments 3 to 5 ml blood was drawn anaerobically in a 5 ml Luer Lok syringe The dead space of the syringes varied from 0.110 to 0 125 ml, and was filled beforehand with 5 / heparin solution The capped syringe was immediately placed in ice water

We have used the 5 / heparin solution because with more diluted solutions clotting occasionally occurred in the concentrated blood obtained during *in vivo* ultrafiltration

All measurements were done on whole blood and within 2 hours of sampling, after vigorous swirling of the syringe to resuspend the erythrocytes We repeatedly measured the decrease in pH occurring during storage in ice water and always found it less than 0 010 after 2 hours.

As the measurement of all samples of one *in vivo* ultrafiltration experiment took up to 100 minutes the first sample was, as a rule, redetermined at the end of the series. The decrease in pH was never more than 0 005 pH unit.

In the dialysis experiments the samples were also drawn anaerobically but they were stored at room temperature for up to 3 hours No change in pH was observed in this period

Apparatus and buffer solutions

The pH was measured with the thermostatted micro glass electrode (G 297/G) and the saturated KCl-calomel electrode (K 497) of Radiometer using the A–C operated direct reading pH meter type PHM 22 with external meter of the same firm (reading precision 0.002 pH units) The two electrodes are connected by a free diffusion junction which is also thermostatted The temperature inside the water jacket of the micro electrode was kept at 37 5 C (± 0 1°) For a detailed description of the electrode chain the reader is referred to SIGGAARD ANDERSEN *et al* (1960)

Two phosphate buffers were used. To the first a pH value of 7 100 was assigned (recipe KH_2PO_4 1 450 g Na_2HPO_4 $2H_2O$ 4 030 g distilled water to 500 ml). The second was considered to have a pH value of 7 400 (recipe KH_2PO_4 0.8487 g Na_2HPO_4 $2H_2O$ 4 830 g distilled water to 500 ml) The recipes were taken from VAN SLYKE *et al* (1949), but slightly modified in that in our case the final concentration of both buffer solutions was 0 0668 M/L instead of 0 0640 M/L.

The slight increase in concentration was found to result in lowering the pH by approximately 0.003 The pH values assigned to the 0 064 molar phosphate buffers by VAN SLYKE *et al.* (1949) are of the Mac Innes convention, which defines the pH scale 0 015 unit lower than the National Bureau of Standards (BATES 1954). Consequently we measured at 37.5°C a pH value of 6.825 for the 0.025 equimolal phosphate buffer which, at a temperature of 38°C, has been accorded a pH value of 6.840 in the NBS scale (BATES 1954).

HCO_3

This was calculated from total CO_2 content and pH using the following equation (PETERS & VAN SLYKE 1932)

$$[HCO_3] = [\text{total } CO_2] \times \frac{1}{1 + \text{antilog}\,(pK_1' - pH)}$$

The value 6.10 was accepted for pK_1 in all *in vivo* ultrafiltration experiments and in the dialysis experiments in which the pH was higher than 7.10. If it was lower pK_1 was corrected for the influence of pH using the values given by SEVERINGHAUS *et al* (1956[a]).

The pK in the above value is in our case in reality pK (see SIGGAARD ANDERSEN 1962, 1963) for which the value of 6.10 seems the best approximation at 38°C and pH 7.40 (NBS scale). Since our pH scale is about 0.015 lower we presumably make negative error in the calculation of HCO_3 by inserting the value of 6.10 for pK_1. However at normal plasma pH and CO_2 content this error would make us underestimate HCO_3 by less than 0.05 mEq/L and it can therefore be neglected in the *in vivo* ultrafiltration experiments. I the dialysis experiments where pH values lower than 6.90 were observed, the error must have been larger, *g* about 0.08 mEq/L at pH 6.90 and about 0.1 mEq/L at pH 6.33 (which was the lowest pH value obtained). Since a systematic error in pH or in pK will affect the calculation of the HCO_3 concentration most at low pH values, we have corrected pK for pH influence in the experiments in which the pH value was below 7.10

In the *in vivo* ultrafiltration experiments initially both total CO_2 and pH were determined for each sample, but later when the trend of pH during venous congestion had been established pH was only determined for every other sample. Thus it was necessary to calculate the pH of the intermediate samples by interpolation but since in the *in vivo* ultrafiltration experiments the difference in pH was never more than 0.02 pH unit for two consecutive samples, the calculated pH must have been correct to within ± 0.01 pH unit. In the dialysis experiments the pH was determined for every sample.

Systematic error

The above mentioned equation is a restatement of the formula of Henderson-Hasselbalch in terms of total CO_2, HCO_3, pK_1 and pH. It is based on the assumption that total CO_2 is made up by HCO_3, H_2CO_3 and physically dissolved CO_2. If CO_2 is present in another chemical combination this would cause an error in the calculation of HCO_3. Two possible substances must be considered

(a) *Carbonate*. In the physiological pH range the amount of carbonate present is very small. At pH 7.80, 38 C and a HCO_3 concentration of 30 mM/L, it can be estimated at 0.3 mM/L in a NaCl + $NaHCO_3$ solution with ionic strength 0.15, if we accept $k_2 = 10^{-10}$ (HASTINGS & SENDROY 1925). Neglect of the presence of carbonate will therefore cause the contribution of CO_2 derivatives to the anionic valencies to be underestimated by 0.3 mEq/L when the pH of the plasma is 7.80. At pH 7.40 the underestimate will be about 0.1 mEq/L and at pH values below 7.00 it will be less than 0.05 mEq/L.

(b) *Carbamat*. It has been shown that in solutions containing amino acids or protein CO_2 can react directly with $-NH_2$ groups to form a carbamino-compound. The reaction can be written as $RNH_2 + CO_2 \rightleftharpoons RNHCOO^- + H^+$. As $-NH_3^+$ does not react with CO_2, carbamate formation occurs only at pH values higher than 6.0, and increases with increasing pH (FAURHOLT 1925, STADIE & O'BRIEN 1936). The reaction has been studied extensively in the case of Hb and HbO_2, which proteins show a rather large

Systematic error

(*a*) EFFECT OF HEPARIN ADDITION – The heparin solution used (Thromboliquine Organon) contains per ml circa 50 mg neutralised sodium heparinate We found its pH about 6 8 (at 38 C) and the addition of increasing amounts of the heparin solution to serum did not affect the pH of the latter significantly unless more than 0 2 ml was added per ml serum. Fig. 17 indicates that at the ratio used in the *in vivo* ultrafiltration experiments (*i.e* about 0 03–0 05 ml heparin solution per ml plasma) there should be no decrease in pH possibly a slight increase (less than 0 004)

Additional experiments were done with whole blood

Ten ml was drawn in a syringe containing 0 02 ml undiluted Thromboliquine, which was mixed with the blood by means of the air bubble contained in the dead space of the syringe. After expelling the air the capped syringe was put in ice water A 5 ml Luer Lok syringe, its dead space filled with undiluted Thromboliquine, was also placed in ice water After about 10 minutes 4 ml blood was transferred anaerobically from the 10 ml syringe into the 5 ml syringe, and both again placed in ice water The pH of their contents was then measured alternatingly for about an hour the syringes being kept in ice water between measurements.

Two experiments were done in which no clotting of the blood occurred. In the first experiment the average pH values were 7 312 and 7.311 in the second experiment 7 371 and 7 370 (the first value refers to the 10 ml syringe) No significant difference in pH was therefore observed although the 10 ml syringe contained 0 002 ml Thromboliquine/ml blood and the 5 ml syringe 0 032 ml Thromboliquine/ml blood. We conclude *that no systematic error in pH measurements* resulted from the use of the undiluted heparin solution

(*b*) STORAGE IN ICE WATER – As a result the pH values obtained in the *in vivo* ultrafiltration experiments may have been somewhat too low (between 0 000 and 0 008 pH unit)

(*c*) CORRECTION FOR SUSPENSION EFFECT OF ERYTHROCYTES – We applied an average correction based solely on the experiments of Severinghaus *et al* We do not know whether for pathological blood the magnitude of the correction should be different

(*d*) pH SCALE – Our pH values are based on the Mac Innes scale since this was apparently the one which Van Slyke *et al* used in their experiments. To convert our values to the NBS scale 0 015 should be added

(*e*) TEMPERATURE OF BLOOD AT THE MOMENT OF SAMPLING – The pH measurements have been done at 37 5 C If the temperature of the venous blood is in reality lower a systematic error is introduced *This error will be considered in* chapter IV

Accidental error

As the difference between duplicate determinations was never more than 0 005 the SE of the mean of duplicate determinations was less than that

be calculated that an error of 0.02 in pH (at pH 7.30) will result in an error of 0.2 / in the calculation of bicarbonate concentration. Since in part of the *in vivo* ultrafiltration experiments about 50 / of the pH values have been calculated by interpolation, with a possible error of ±0.01 pH unit, the SE of the mean of a duplicate determination presumably is somewhat larger for HCO_3 than for total CO_2 but it seems unlikely that it is more than 0.20 mEq/L. At pH values below 7.00 however the spread must have been somewhat larger

Partial carbon dioxide tension (P_{CO_2}) in plasma

As we were only interested in the magnitude of the relative changes observed during *in vivo* ultrafiltration we thought it sufficient to estimate P_{CO_2} from total CO_2 content and pH by means of the line chart published by VAN SLYKE & SENDROY (1928). The original graph was used as we learned from MOLLER (1959) that the reproduction of the graph in PETERS & VAN SLYKE (1932) is erroneous.

Inorganic phosphate

This was determined as described by DE VRIES in GORTER & DE GRAAFF (1955). After deproteinisation, complex-ion formation with molybdic acid in the presence of ferrous ions is measured colorimetrically at 660–720 mμ, and compared with standard solutions (2.5 mg / and 5.0 mg / P). The maximum difference between duplicate analyses in our series was 0.1 mg .

Lactate

The method of BARKER & SUMMERSON (1941) was used. The samples were obtained as follows 2 ml blood was drawn in a dry syringe and immediately ejected into a glass tube containing 10 μl of 5 heparin solution After mixing, 0.2 ml was pipetted into a glass tube containing an acid cadmium sulfate solution and after vigorous shaking the tube was put in ice To prevent contamination from the skin the tubes were kept closed with aluminium foil All determinations were done within 3 hours after sample taking. Racemic lactate was used for preparing standards of 0.50, 1.00 2.00 and 3.00 mM/L. With each series of determinations the four standards were run and a blank. The maximum difference between duplicate determinations was 0.1 mM/L, the mean difference 0.01 mM/L.

Haemoglobin

The alkaline haematin method was used (GORTER & DE GRAAFF 1955). The O.D. scale is calibrated against total blood iron (GORTER & DE GRAAFF 1955) by multiplying the iron concentration (in mg) with 0.3 Hb concentration (in g) is obtained The maximum difference between duplicate analyses was 0.2 g .

Hb oxygen saturation ($HbO_{2\%}$)

We used the two-wavelength spectrophotometric method as described by ZIJLSTRA & MULLER (1957), the blood having a layer thickness of 0.013 cm. Following a suggestion of ZIJLSTRA (1958) O.D. was read at 560 mμ and 522 mμ, a Unicam SP 500 spectrophotometer being used The percentage oxygen saturation is calculated from the

tendency to carbamate formation especially Hb (HENRIQUES, 1928 MARGARIA, 1931 FERGUSON & ROUGHTON 1934[1] 1934[2] ROUGHTON 1935 STADIE & O BRIEN 1937 WYMAN 1948 ROUGHTON 1954) In the case of the plasma proteins this tendency is very much smaller As direct determination of carbamino-compound in plasma has not been possible (ROUGHTON 1962) STADIE & O BRIEN (1937) used an indirect method. But the value at which they arrive for plasma at physiological pH 38°C and normal P_{CO_2} (*i e* 0.3 to 0 5 mM/L CO_2 *bound as carbamate*) *is extrapolated from experiments* at pH 9 0 and 0 C with a solution containing 30 / horse serum protein It should be mentioned that Stadie and O Brien s values for Hb and HbO_2 carbamate at physiological conditions are about 50 / higher than those found by Ferguson and Roughton who used a more direct determination In a personal communication Prof ROUGHTON (1962) has given as his opinion that under physiological conditions 0 5 mM/L must be considered as a maximal value for plasma carbamate. The value is probably smaller since GIUSTINA *et al* (1953) concluded from direct determinations on solutions of serumalbumin that at pH values lower than 8 0 at a temperature of 22 C and at physiological P_{CO_2} the amount of albumin carbamate is negligible The ε NH_2 groups cannot form carbamate to any extent at physiological pH (ROUGHTON 1954), so that it must be assumed that under these conditions only α NH_2 groups *i.e* terminal amino acids of the protein molecule react with CO_2 Since in normal plasma the number of albumin molecules per unit volume is much higher than that of the molecules of other proteins, it is reasonable to assume that the findings of Giustina *et al* preclude that in normal plasma more then 0 2 mM/L of total CO_2 is bound as carbamate.

We have discussed the possible existence of plasma carbamate in some detail, because when larger amounts of the latter are present a significant error will be made in the interpretation of our experimental results. Carbamate represents a negative charge on the protein molecule, and by mistaking it for HCO_3 the negative valency of the plasma proteins is underestimated. Moreover Stadie and O Brien (1937) found both for Hb and HbO_2 a positive linear correlation between protein concentration and carbamate concentration. A similar relation presumably exists in the case of other proteins, and during *in vivo* ultrafiltration any error caused by mistaking carbamate for HCO_3 will therefore increase.

On the strength of the arguments put forward we shall assume that the error due to neglecting the existence of carbamate in plasma will lead to overestimating HCO_3 concentration by 0 2 mEq/L plasma at pH 7 40 At higher pH values the error should become larger below pH 7 00 it should be negligible (see effect of pH on carbamino-albumin in the publication by GIUSTINA *et al*)

The errors due to neglecting the presence of carbonate and the presence of carbamino-protein tend to cancel each other From the estimates made above we conclude that the combined effect of the two errors on the calculation of HCO_3 will be negligible at pH values below 7 00 but that it will result in overestimating HCO_3 by 0 1 mEq/L plasma at pH 7 40 and by about 0 2 mEq/L plasma at pH 7 80 The error increases when the protein concentration increases

Accidental error

The SE of the mean of two duplicate determinations was found to be 0 14 mM/L for the total CO_2 determination whereas the maximum difference between duplicates was 0 005 for the pH determination. Assuming a value of 30 0 mM/L for total CO_2 it can

be calculated that an error of 0.02 in pH (at pH 7.30) will result in an error of 0.2 % in the calculation of bicarbonate concentration. Since in part of the *in vivo* ultrafiltration experiments about 50 % of the pH values have been calculated by interpolation, with a possible error of ±0.01 pH unit, the SE of the mean of a duplicate determination presumably is somewhat larger for HCO_3 than for total CO_2 but it seems unlikely that it is more than 0.20 mEq/L. At pH values below 7.00 however the spread must have been somewhat larger.

Partial carbon dioxide tension (P_{CO_2}) in plasma

As we were only interested in the magnitude of the relative changes observed during *in vivo* ultrafiltration, we thought it sufficient to estimate P_{CO_2} from total CO_2 content and pH by means of the line chart published by VAN SLYKE & SENDROY (1928). The original graph was used as we learned from MOLLER (1959) that the reproduction of the graph in PETERS & VAN SLYKE (1932) is erroneous.

Inorganic phosphate

This was determined as described by DE VRIES in GORTER & DE GRAAFF (1955). After deproteinisation, complex-ion formation with molybdic acid in the presence of ferrous ions is measured colorimetrically at 660–720 mμ, and compared with standard solutions (2.5 mg % and 5.0 mg % P). The maximum difference between duplicate analyses in our series was 0.1 mg %.

Lactate

The method of BARKER & SUMMERSON (1941) was used. The samples were obtained as follows: 2 ml blood was drawn in a dry syringe and immediately ejected into a glass tube containing 10 μl of 5 % heparin solution. After mixing, 0.2 ml was pipetted into a glass tube containing an acid cadmium sulfate solution, and after vigorous shaking the tube was put in ice. To prevent contamination from the skin the tubes were kept closed with aluminium foil. All determinations were done within 3 hours after sample taking. Racemic lactate was used for preparing standards of 0.50, 1.00, 2.00 and 3.00 mM/L. With each series of determinations the four standards were run and a blank. The maximum difference between duplicate determinations was 0.1 mM/L, the mean difference 0.01 mM/L.

Haemoglobin

The alkaline haematin method was used (GORTER & DE GRAAFF 1955). The O.D. scale is calibrated against total blood iron (GORTER & DE GRAAFF 1955); by multiplying the iron concentration (in mg %) with 0.3 Hb concentration (in g %) is obtained. The maximum difference between duplicate analyses was 0.2 g %.

Hb oxygen saturation (HbO_2%)

We used the two-wave length spectrophotometric method as described by ZIJLSTRA & MULLER (1957), the blood having a layer thickness of 0.013 cm. Following a suggestion of ZIJLSTRA (1958) O.D. was read at 560 mμ and 522 mμ, a Unicam SP 500 spectrophotometer being used. The percentage oxygen saturation is calculated from the

tendency to carbamate formation especially Hb (HENRIQUES 1928 MARGARIA, 1931 FERGUSON & ROUGHTON 1934[1] 1934[2] ROUGHTON 1935 STADIE & O BRIEN 1937 WYMAN 1948 ROUGHTON 1954) In the case of the plasma proteins this tendency is very much smaller As direct determination of carbamino-compound in plasma has not been possible (ROUGHTON 1962) STADIE & O BRIEN (1937) used an indirect method. But the value at which they arrive for plasma at physiological pH 38 C and normal P_{CO_2} (*i.e* 0 3 to 0.5 mM/L CO_2 bound as carbamate) is extrapolated from experiments at pH 9 0 and 0 C with a solution containing 30 / horse serum protein It should be mentioned that Stadie and O Brien s values for Hb and HbO_2 carbamate at physiological conditions are about 50 / higher than those found by Ferguson and Roughton who used a more direct determination In a personal communication Prof ROUGHTON (1962) has given as his opinion that under physiological conditions 0 5 mM/L must be considered as a maximal value for plasma carbamate The value is probably smaller since GIUSTINA *et al* (1953) concluded from direct determinations on solutions of serumalbumin that at pH values lower than 8 0 at a temperature of 22 C and at physiological P_{CO_2} the amount of albumin carbamate is negligible The ε NH_2 groups cannot form carbamate to any extent at physiological pH (ROUGHTON 1954), so that it must be assumed that under these conditions only α NH_2 groups *i.e* terminal amino acids of the protein molecule react with CO_2 Since in normal plasma the number of albumin molecules per unit volume is much higher than that of the molecules of other proteins, it is reasonable to assume that the findings of Giustina *et al* preclude that in normal plasma more then 0 2 mM/L of total CO_2 is bound as carbamate

We have discussed the possible existence of plasma carbamate in some detail, because when larger amounts of the latter are present a significant error will be made in the interpretation of our experimental results Carbamate represents a negative charge on the protein molecule, and by mistaking it for HCO_3 the negative valency of the plasma proteins is underestimated. Moreover Stadie and O Brien (1937) found both for Hb and HbO_2 a positive linear correlation between protein concentration and carbamate concentration A similar relation presumably exists in the case of other proteins, and during *in vivo* ultrafiltration any error caused by mistaking carbamate for HCO_3 will therefore increase.

On the strength of the arguments put forward we shall assume that the error due to neglecting the existence of carbamate in plasma will lead to overestimating HCO_3 concentration by 0 2 mEq/L plasma at pH 7 40 At higher pH values the error should become larger below pH 7 00 it should be negligible (see effect of pH on carbamino-albumin in the publication by GIUSTINA *et al*)

The errors due to neglecting the presence of carbonate and the presence of carbamino-protein tend to cancel each other From the estimates made above we conclude that the combined effect of the two errors on the calculation of HCO_3 will be negligible at pH values below 7 00 but that it will result in overestimating HCO_3 by 0 1 mEq/L plasma at pH 7 40 and by about 0 2 mEq/L plasma at pH 7 80 The error increases when the protein concentration increases

Accidental error

The SE of the mean of two duplicate determinations was found to be 0 14 mM/L for the total CO_2 determination whereas the maximum difference between duplicates was 0 005 for the pH determination Assuming a value of 30.0 mM/L for total CO_2 it can

our hands the latter gives values which are on the average 2 % lower but the correlation is a linear one and we may conclude that our data on Hb saturation are reliable to within 3 % over the range 20 to 100 % HbO_2.



Summary of chapter III

Since we will determine NCE by correlating changes in protein concentration with changes in (cat min Cl + HCO_3) the following conclusions are relevant

The plasma obtained in our experiments was true plasma but for the presence of a known amount of heparin solution. For this a correction can be made.

A small systematic error was found to exist in the determination of Na, K and HCO_3. For Na and K the error was negative and of the order of respectively 0.07 % and 2 % in normal plasma. For HCO_3 it was positive and of the order of 0.3 % in normal plasma. For all 3 substances the error increases with increasing protein concentration. In the case of HCO_3 it also is pH dependent.

We could find no evidence for a systematic error in the determination of water content of plasma, and of the concentrations of protein, Ca, Mg and Cl.

The SE of the mean of a duplicate determination as calculated from about 550 duplicate determinations of plasma, was found to be

Na	0.08 mEq/L	Cl	0.08 mEq/L
K	0.008 mEq/L	HCO_3	0.20 mEq/L
Ca + Mg	0.012 mEq/L		

For (cat min Cl + HCO_3), which is calculated from the means of the duplicate determinations of the above mentioned ions, the SD should therefore be

$$\sqrt{0.08^2 + 0.008^2 + 0.012^2 + 0.08^2 + 0.20^2} = 0.25 \ mEq/L.$$

Thus accidental error is independent of protein concentration. The same is true for the error in protein determination, for which the SE of the mean of a duplicate analysis was found to be 0.3 g/L plasma.

It is concluded that the systematic and accidental errors of the analytical techniques are sufficiently small to permit the determination of NCE in the manner to be described in the next chapters.

formula

$$HbO_2\,\% = \left(a\ \frac{O\,D_{560}}{O\,D_{522}} - b\right)\ 100,$$

in which O D indicates optical density at the wavelength indicated and a and b are constants which are calculated from a series of measurements of $O\,D_{560}/O\,D_{522}$ on solutions containing exclusively Hb or HbO_2 We refer to ZIJLSTRA & MULLER (1957) for the argumentation

We could corroborate the mean values given by Zijlstra for the constants a (3.081) and b (1 623) (ZIJLSTRA 1958), but in our hands the spread was fairly large. This is due to the steepness of both absorption curves at the 522 mµ isobestic point. A deviation of 0.5 mµ from the true isobestic point leads to a 2 % error in calculating HbO_2 %. Fortunately over the range 520 to 524 mµ the error involved has the same direction and magnitude for HbO_2 and for Hb. We have therefore adopted the following procedure O D was measured at 520 522, 524 mµ and at 558, 560, 562 mµ both directly and after saturation of the blood sample with O_2 in a tonometer Then HbO_2 % of the saturated sample was calculated from

$$\left(3.081\ \frac{O\,D_{560}}{O.D_{522}} - 1\,623\right)\ 100.$$

Generally 100 % ± 1 was found, but if a lower or higher value was obtained, that wavelength was accepted as isobestic point, the O D of which gave a value of 100 % when inserted in the abovementioned equation. The same wavelength was then used for calculating the Hb saturation of the original samples, the precise O D being obtained by interpolation from the O.D measured at the six wavelengths mentioned above. With this indirect manner of checking, the apparent isobestic wavelength was found to vary between 522 and 523 mµ. When proceeding as described above, we found the duplicate determinations equal to within 2 %.

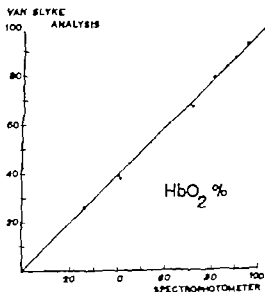


Fig. 18 Determination of percentage HbO Comparison of manometric method with spectrophotometric method.

Fig 18 shows the correlation between percentage HbO_2 determined in the manner described and that obtained with the manometric method of Van Slyke and Neill In

our hands the latter gives values which are on the average 2 % lower but the correlation is a linear one and we may conclude that our data on Hb saturation are reliable to within 3 % over the range 20 to 100 % HbO_2.



Summary of chapter III

Since we will determine NCE by correlating changes in protein concentration with changes in (cat min Cl + HCO_3) the following conclusions are relevant

The plasma obtained in our experiments was true plasma but for the presence of a known amount of heparin solution. For this a correction can be made.

A small systematic error was found to exist in the determination of Na, K and HCO_3. For Na and K the error was negative and of the order of respectively 0.07 % and 2 % in normal plasma. For HCO_3 it was positive and of the order of 0.3 % in normal plasma. For all 3 substances the error increases with increasing protein concentration. In the case of HCO_3 it also is pH dependent.

We could find no evidence for a systematic error in the determination of water content of plasma, and of the concentrations of protein, Ca, Mg and Cl

The SE of the mean of a duplicate determination, as calculated from about 550 duplicate determinations of plasma, was found to be

Na	0.08 mEq/L	Cl	0.08 mEq/L
K	0.008 mEq/L	HCO_3	0.20 mEq/L
Ca + Mg	0.012 mEq/L		

For (cat min Cl + HCO_3), which is calculated from the means of the duplicate determinations of the above mentioned ions, the SD should therefore be

$$\sqrt{0.08^2 + 0.008^2 + 0.012^2 + 0.08^2 + 0.20^2} = 0.25 \text{ mEq/L}.$$

This accidental error is independent of protein concentration. The same is true for the error in protein determination, for which the SE of the mean of a duplicate analysis was found to be 0.3 g/L plasma.

It is concluded that the systematic and accidental errors of the analytical techniques are sufficiently small to permit the determination of NCE in the manner to be described in the next chapters.

In vivo ultrafiltration

During his investigation of haemoglobin concentration as an indicator of changes in plasma volume GERBRANDY (1951) studied the effect of upper arm compression at 90 mm Hg. He became impressed by the magnitude of the increase in protein concentration of the blood taken from a cubital vein and conceived the idea of using this phenomenon for the determination of the protein bound fraction of plasma constituents.

Only recently we learned that the principle has already been used by RECKNAGEL (1930) to demonstrate that bilirubin is bound to plasma proteins, and by VAHLQUIST (1941) to demonstrate the same for serum iron. The latter author actually coined the term *In-vivo-Dialysis*. We also regret that it was not known to us until after the preparation of our manuscript that NIEMIRO already in 1955 published the results of an investigation on the dependence of the calcium content of the blood, in which he determined protein-bound calcium in precisely the same manner as we did at a later date (NIEMIRO 1955). The authors mentioned have made no attempt to study *in vivo* ultrafiltration in more detail.

GERBRANDY *et al.* (1957, 1960) found that during venous congestion, in accordance with expectation, substances not associated with protein (urea, glucose) showed no significant change in concentration per Kg H_2O, whereas the substances known to be completely protein-bound (iron, bilirubin, cholesterol, alkaline phosphatase) showed the same percentage increase as total protein. The percentage increase in Ca and Mg concentration was found to be about 40% of that of protein, the percentage increase in Na concentration about 10% of that of protein. For K this value was near 20%, but after venous congestion K concentration – in contrast to the concentration of the other cations – did not return to its precompression level. It was therefore concluded that the increase in K concentration during venous congestion was partly due to a shift of K from cells (erythrocytes?) to plasma as the result of the concomitant changes in O_2 and CO_2 content of the blood (GERBRANDY *et al.* 1960).

In a later study it was demonstrated that, by prolonging the period of venous congestion up to 35 minutes and increasing the number of samples taken, the method could be refined and used for the determination of protein-bound Ca and Mg in the individual person, provided the congested arm was kept completely relaxed (VAN LEEUWEN *et al.* 1961). Further evidence was presented to substantiate the conclusion that under these conditions factors such as pH, protein composition and lactate formation remain sufficiently constant to warrant the use of the method for the determination of protein bound Ca and Mg. Preliminary results obtained for Na suggested the possibility of using the method for the determination of the NCE of plasma proteins, provided it could be shown that the increase in difference between the sum concentration of the cations and

the sum concentration of Cl and HCO_3 i.e (cat min Cl + HCO_3) observed during controlled venous congestion, is a function of the total protein concentration solely

From earlier experiments and from additional experiments to be reported in this study it was concluded that this is so with certain restrictions. Before considering the evidence for this conclusion (which will entail a study of the effect of upper arm compression on the forearm) we will first describe the technical procedure as used during this study and the manner in which NCE and protein-bound Na, Ca and Mg were calculated from the experimental data.

IN VIVO ULTRAFILTRATION, TECHNIQUE AND CALCULATIONS

Technique

All experiments were done between 8.30 and 10.30 a.m. The subjects had not eaten since the evening before, but they were allowed one cup of tea (± 150 ml), with about 10 g of sugar at about 7.30 a.m. The normal subjects and the out-patients were instructed to avoid physical exercise as much as possible in the hours preceding the investigation.

On arrival the subject was comfortably placed in a semi-recumbent position with one arm completely relaxed and extended on a cushion. After 15 minutes rest, an 18 gauge needle with pointed stylet was introduced in the median or the ulnar cubital vein of the extended arm following 1 minute compression of the upper arm at 60 mm Hg. The pressure was then immediately released and another 5 minutes were allowed to pass before the first of the *precompression samples* was taken Generally two sometimes more, samples were drawn at 3 minutes intervals. Thereafter the upper arm was compressed by inflating a carefully adjusted manometer cuff

1 most cases the pressure was 100 mm Hg, but in the few instances in which the systolic pressure was lower used the highest pressure at which the radial pulse could still be felt distinctly in the present study the pressure in the cuff ranged from 80 to 100 mm Hg.

While keeping the pressure constant within 5 mm Hg, the *compression samples* were taken. In the normal persons their number varied from 5 to 8, and the first sample was generally drawn 5 to 7 minutes after the start of upper arm compression the other samples were taken at intervals of 2 to 3 minutes, after 20 minutes of compression at intervals of 5 minutes or longer In most patients sampling was started after 15 minutes and only 3 to 5 compression samples were taken, in order to minimise blood loss. Finally after 30 to 40 minutes compression, the pressure was abruptly released and 2 to 3 *postcompression samples* were drawn In a number of cases the first was taken within 2 minutes after decompression, but generally 6 minutes were allowed to pass. During the whole period of investigation care was taken that the extended arm remained relaxed.

In the case of the normal subjects the total number of samples was 8 to 13 amounting to a loss of 130 to 220 ml blood, but in the case of the patients – where the total number of samples varied from 6 to 10 – the total amount of blood taken varied from 80 (in case of anaemia) to 180 ml.

The handling of the blood samples has already been described in chapter III The plasma obtained was always analysed for Na, K Ca, Mg, Cl, total CO_2 and total protein. Originally blood pH was determined with every sample, later on with every other sample. In the latter case the pH of the remaining samples was determined by inter

polation so that HCO_3 could be calculated for all samples Haemoglobin and haematocrit were determined in at least one precompression and one compression sample. In practically all experiments inorganic phosphate was determined in one of the precompression samples in a number of experiments also in compression samples. The same applies to the determination of the protein fractions, which were determined according to Majoor and in most cases also by paper electrophoresis In a number of experiments the following analyses were done in one or more samples blood lactate, $HbO_{2}/_{\mu}$ cholesterol creatinine urea. Also in nearly all experiments plasma water content was determined for at least one precompression sample and one sample taken towards the end of the period of compression but generally more samples were analysed a linear relation between total protein concentration and water content of plasma was calculated for each particular experiment and with its aid all analytical values of the experiment were converted to concentrations per Kg/H_2O

Calculations

For each experiment regression lines were calculated for the correlation between the concentration of respectively (cat min $Cl + HCO_3$) Na Ca and Ca + Mg and the

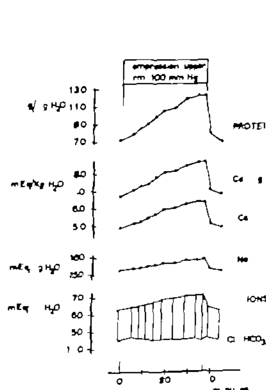


Fig. 19 Changes in concentration as observed in one ultrafiltration experiment.

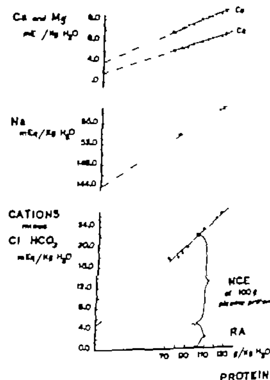


Fig. 20 Graphic representation of manner of calculating NCE and RA (same experiment as fig. 19).

concentration of protein (method of least squares) From the slope of these regression lines we calculated the NCE per 100 g of total protein respectively the amount of cation bound by 100 g of protein By extrapolating to protein *concentration zero* we obtained in the case of (cat min $Cl + HCO_3$) the concentration of RA (rest anions) and in the

case of the individual cations the concentration of cation not associated with protein ("free cation").

The procedure is graphically represented in fig. 19 and 20. Fig. 19 shows the changes in the concentration of the ions and of protein as observed in one *in vivo* ultrafiltration experiment, and fig. 20 the correlation between the changes in protein concentration and in ion concentration for the same experiment.

A STUDY OF THE SUBJECTIVE AND OBJECTIVE EFFECTS OF PROLONGED COMPRESSION OF THE UPPER ARM

By treating the experimental data in the manner just described, we tacitly assume that during the experiment the concentration of protein is the sole determinant of the concentration of the cations and the sum concentration of Cl and HCO_3.

For this to be so the plasma samples obtained by means of *in vivo* ultrafiltration must fulfill the following conditions

(1) The correlation between the concentration of a cation e.q. (cat min Cl + HCO_3) and of plasma protein is linear for protein concentrations ranging from 0 to about 130 g/Kg H_2O

(2) The protein composition remains unaltered.

(3) Protein-protein interaction can be neglected.

(4) The concentration of RA does not change.

(5) The effect of the Donnan equilibrium can be neglected

(6) The pH remains constant.

In order to decide whether these conditions indeed exist, we will have to consider in some detail the changes which occur in the forearm during venous compression We will limit ourselves to the factors which might influence the effectiveness of the *in vivo* ultrafiltration method For a review of existing knowledge concerning the blood kinetics of the congested limb and the permeability of the capillary wall the reader is referred to LANDIS (1934), who with his collaborators has provided much of the present knowledge on these subjects, and to the interesting article by PAPPENHEIMER (1953). A fairly complete bibliography concerning most aspects of haemoconcentration and haemodilution can be found in Gerbrandy's thesis (1951).

Blood flow in, appearance of, and sensations in the forearm during venous congestion
In the resting and relaxed arm the capillary and venous system is not filled to capacity On sudden compression of the upper arm at a pressure somewhat below systolic arterial pressure the in-flowing blood will first be accomodated in this latent vascular reservoir From measurements done by GERBRANDY (1951) it can be seen that as a result of this buffer effect the pressure in the median cubital vein rises only gradually until – after about 5 minutes it surpasses the compression pressure, and venous blood starts to lea e the forearm again. The increase in venous and in capillary pressure has the effect both of increasing ultrafiltration and of decreasing blood flow The latter phenomenon is the unavoidable corollary of the decrease in arterio-venous pressure gradient. The effect which increasing the venous pressure to 60 mm Hg has on forearm blood flow was studied by several workers w th the aid of plethysmography FISCH *et al* (1950) calculated from their own results and those of HALPERIN *et al.* (1948) a linear relation

which appeared to indicate that at venous pressures higher than 80 mm Hg blood flow would become zero Actually their own data indicate that the relation is not a linear one. Furthermore the fact that we could compress the upper arm at a pressure of 100–110 mm Hg for periods up to 40 minutes without signs of anaerobic metabolism clearly indicates that even at those high venous pressures some flow must persist. We could not measure the magnitude of the latter with the plethysmographic occlusion method because at 100 mm Hg the venous pressure is too near the systolic arterial pressure. However Dr J Van Noordwijk and Dr T Oei (from the department of Pharmacology University of Amsterdam) were kind enough to determine the blood flow in a relaxed forearm before, during and after compression of the upper arm at 60 mm Hg. One experiment was done and the results are shown in fig. 21

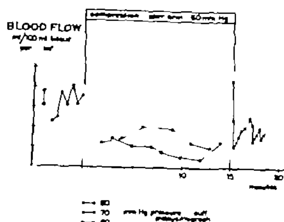


Fig. 21 – Blood flow through forearm before, during and after compression of upper arm (one experiment) See text for explanation.

The subject s systolic arterial pressure was 100 mm Hg. Before compression the collecting pressure in the plethysmograph cuff was 80 mm Hg, after compression it was 70 mm Hg, but during compression two collecting pressures were used alternately in the plethysmograph cuff, to w t 60 and 80 mm Hg. From fig. 21 it will be evident that the intermittent application of the collecting pressure affects the results of the blood flow measurement since a higher blood flow is obtained with the lower collecting pressure.

Although the absolute values obtained for the blood flow are therefore of doubtful significance fig. 21 may serve to indicate the trend, *i e* a decrease in blood flow during upper arm compression which levels off after about 5 minutes, with immediately after decompression a sudden and short lasting overshoot this is followed by a longer period in which the blood flow is definitely lower than in the precompression period The circulatory changes have a rather pronounced effect on the appearance of the congested limb A detailed description has already been given by AUSPITZ (1874) and later by BIER (1906) and DAUTREBANDE *et al* (1923) Our own observations can be described as follows

In the first minutes of compression a swelli g of the superficial veins is seen, followed by an increasingly intense cyanosis of the skin. The latter acquires a mottled appearance with peculiar red patches showing against a bluish background After about 10 minutes the cyanosis does not further increase, and gradually some edema can be detected. Subjectively the sensation of 'swell ng' is most disagreeable between 3 and 10 minutes after the start of compression, when t is often accompanied by itching. Gradually a dulling of the senses occurs, until the arm feels like a corpus alienum occasionally a remnant of the itching sensations reminds the owner that he is still in possession. After about 20 minutes a paresis develops and I the few instances in which we have

continued the compression for 30 minutes the forearm and hand muscles were completely paralysed. A similar observation was made by DAUTREBANDE *et al.* (1923) during their experimental study of circulatory stasis. Immediately after decompression the swollen veins are seen to collapse and the skin regains a healthy rosy colour within 2 minutes. But thereafter a slight blanching, especially of the fingers, becomes perceptible which may persist for 10 to 15 minutes. Subjectively the first three minutes after decompression are the most unpleasant of the whole experiment. first a hot, tingling sensation creeps through hand and forearm, but this is followed by a peculiar cramped feeling in the muscles of the forearm and it needs some force of will to keep the arm and hand relaxed in this period. This lasts for about 2 minutes and then all abnormal sensations disappear. As regards the paresis, even in the few experiments in which paralysis was induced by prolonging the period of compression, the hand and forearm could be moved without any difficulty within one minute after decompression. Presumably muscle paresis during, and the cramped feeling after compression are the result of pressure on the n. brachialis and n. radialis in the upper arm.

In the more than 120 experiments in which prolonged upper arm compression was applied, Trousseau's sign was only seen twice: once in a patient with hypoparathyroidism and a 'free' calcium concentration of 2.0 mEq/Kg H_2O and here it lasted only one minute and vanished during the continuation of the compression; the other subject was a healthy young male with completely normal blood chemistry in whom a similar short-lasting Trousseau phenomenon developed during compression. It was never seen in the hypocalcaemic uraemic patients, some of whom had 'free' calcium concentrations lower than 1.6 mEq/Kg H_2O.

A rather pronounced petechial rash developed in about 40% of the subjects, both in healthy persons and in patients. But contrary to expectation we never saw it in uraemic patients, and in patients with multiple myeloma only to a slight degree. In about 5% of all cases it was very marked, and these were all healthy men. Moreover, the tendency to develop a petechial rash was a personal characteristic, since the same persons could always be relied upon to produce a spectacular rash for the benefit of the medical students. The formation of petechiae may have some bearing on the observations concerning protein leakage from the vascular bed during venous congestion (see later).

Finally it should be mentioned that although in some subjects the experiment was often repeated, always using the same arm and sometimes prolonging compression up to 50 minutes, no signs of venous thrombosis were ever observed. Nevertheless, the conditions of the experiment are such that this would appear to be a potential danger. In a few preliminary experiments DEN OTTOLANDER observed an increase in practically all the clotting factors parallel with protein concentration. But on further investigation it was found that the fibrinolytic activity of the venous blood increased even more during compression (DEN OTTOLANDER 1962). Furthermore, Bier, who in the beginning of this century introduced prolonged venous congestion as a method of treating chronic inflammatory conditions, did not mention an increased incidence of thrombosis following this treatment nor, indeed, any other untoward reaction (BIER 1906). These observations appear to justify the conclusion that no complications are to be feared.

Considering the sensations described above, the method was remarkably well tolerated, especially by the patients. It is, however, important to explain why the colour of the arm changes, what the duration of the various sensations will be and to engage the subject in a discussion which has his particular interest. At times he is visibly reassured by learning that the investigator underwent the same operation several times and obviously still is in full possession of both his arm and his health. It must be stressed

which appeared to indicate that at venous pressures higher than 80 mm Hg blood flow would become zero Actually their own data indicate that the relation is not a linear one. Furthermore, the fact that we could compress the upper arm at a pressure of 100–110 mm Hg for periods up to 40 minutes without signs of anaerobic metabolism clearly indicates that even at those high venous pressures some flow must persist. We could not measure the magnitude of the latter with the plethysmographic occlusion method because at 100 mm Hg the venous pressure is too near the systolic arterial pressure However Dr L Van Noordwijk and Dr T Oei (from the department of Pharmacology University of Amsterdam) were kind enough to determine the blood flow in a relaxed forearm before, during and after compression of the upper arm at 60 mm Hg. One experiment was done and the results are shown in fig. 21

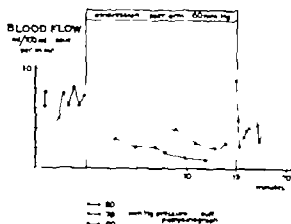


Fig. 21 Blood flow through forearm before, during and after compression of upper arm (one experiment). See text for explanation.

The subject s systolic arterial pressure was 100 mm Hg. Before compression the collecting pressure in the plethysmograph cuff was 80 mm Hg, after compression it was 70 mm Hg, but during compression two collecting pressures were used alternately in the plethysmograph cuff, to wit 60 and 80 *mm Hg*. From fig. 21 it will be evident that the intermittent application of the collecting pressure affects the results of the blood flow measurement since a higher blood flow is obtained with the lower collecting pressure

Although the absolute values obtained for the blood flow are therefore of doubtful significance, fig 21 may serve to indicate the trend, *i e* a decrease in blood flow during upper arm compression which levels off after about 5 minutes, with immediately after decompression a sudden and short lasting overshoot this is followed by a longer period in which the blood flow is definitely lower than in the precompression period. The circulatory changes have a rather pronounced effect on the appearance of the congested limb A detailed description has already been given by AUSPITZ (1874) and later by BIER (1906) and DAUTREBANDE *et al* (1923) *Our own observations can be described as follows*

In the first minutes of compression a swelling of the superficial veins is seen, followed by an increasingly intense cyanosis of the skin. The latter acquires a mottled appearance with peculiar red patches showing against a bluish background. After about 10 minutes the cyanosis does not further increase, and gradually some edema can be detected. Subjectively the sensation of swelling is most disagreeable between 3 and 10 minutes after the start of compression, when it is often accompanied by itching. Gradually a dulling of the senses occurs, until the arm feels like a corpus alienum occasionally a remnant of the itching sensations reminds the owner that he is still in possession. After about 20 minutes a paresis develops and in the few instances in which we have

continued the compression for 50 minutes the forearm and hand muscles were completely paralysed. A similar observation was made by DAUTREBANDE *et al.* (1923) during their experimental study of circulatory stasis. Immediately after decompression the swollen veins are seen to collapse and the skin regains a healthy rosy colour within 2 minutes. But thereafter a slight blanching, especially of the fingers, becomes perceptible which may persist for 10 to 15 minutes. Subjectively the first three minutes after decompression are the most unpleasant of the whole experiment. At first a hot, tingling sensation creeps through hand and forearm, but this is followed by a peculiar cramped feeling in the muscles of the forearm and it needs some force of will to keep the arm and hand relaxed in this period. This lasts for about 2 minutes and then all abnormal sensations disappear. As regards the paresis, even in the few experiments in which a paralysis was induced by prolonging the period of compression, the hand and forearm could be moved without any difficulty within one minute after decompression. Presumably muscle paresis during, and the cramped feeling after compression are the result of pressure on the n. brachialis and n. radialis in the upper arm.

In the more than 120 experiments in which prolonged upper arm compression was applied Trousseau's sign was only seen twice: once in a patient with hypoparathyroidism and a 'free' calcium concentration of 2.0 mEq/Kg H_2O and here it lasted only one minute and vanished during the continuation of the compression; the other subject was a healthy young male with completely normal blood chemistry in whom a similar short-lasting Trousseau phenomenon developed during compression. It was never seen in the hypocalcaemic uraemic patients, some of whom had 'free' calcium concentrations lower than 1.6 mEq/Kg H_2O.

A rather pronounced petechial rash developed in about 40 % of the subjects, both in healthy persons and in patients. But contrary to expectation we never saw it in uraemic patients, and in patients with multiple myeloma only to a slight degree. In about 5 % of all cases it was very marked, and these were all healthy men. Moreover the tendency to develop a petechial rash was a personal characteristic, since the same persons could always be relied upon to produce a spectacular rash for the benefit of the medical students. The formation of petechiae may have some bearing on the observations concerning protein leakage from the vascular bed during venous congestion (see later).

Finally it should be mentioned that although in some subjects the experiment was often repeated, always using the same arm and sometimes prolonging compression up to 50 minutes, no signs of venous thrombosis were ever observed. Nevertheless, the conditions of the experiment are such that this would appear to be a potential danger. In a few preliminary experiments DEN OTTOLANDER observed an increase in practically all the clotting factors parallel with protein concentration. But on further investigation it was found that the fibrinolytic activity of the venous blood increased even more during compression (DEN OTTOLANDER 1962). Furthermore, BIER who in the beginning of this century introduced prolonged venous congestion as a method of treating chronic inflammatory conditions, did not mention an increased incidence of thrombosis following this treatment nor indeed any other untoward reaction (BIER 1906). These observations appear to justify the conclusion that no complications are to be feared.

Considering the sensations described above, the method was remarkably well tolerated, especially by the patients. It is, however, important to explain why the colour of the arm changes, what the duration of the various sensations will be and to engage the subject in a discussion which has his particular interest. At times he is visibly reassured by learning that the investigator underwent the same operation several times and obviously still is in full possession of both his arm and his health. It must be stressed

that it is essential to supervise continuously the experimental arm and to ensure its continued relaxation Only in this way can it be assumed that tissue metabolism remains at a minimum during the whole period of the experiment.

The change in protein concentration during compression

In order to obtain a reasonably uniform distribution of experimental points over the range of protein concentrations it was necessary to study the relation between the duration of compression and the increase in protein concentration.

As mentioned before it takes some time before in the relaxed arm the venous pressure exceeds the compression pressure and it is to be expected that the protein concentration

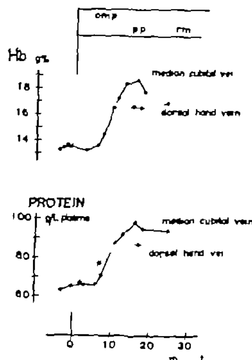


Fig. 22 Changes in Hb- and protein concentration in venous blood sampled from 2 different forearm veins during compression.

will not increase immediately after starting the compression of the upper arm Moreover the various regions of the vascular bed should differ in this respect Fig. 22 shows that this is so The vascular area draining via the dorsal hand vein (mainly skin) has a relatively small latent vascular capacity As a result the capillary pressure will rise immediately after the start of compression On the other hand the muscular area from which the median cubital vein receives most of its blood has a large latent vascular capacity with – in the relaxed arm – presumably a rather low tissue pressure (GERBRANDY 1951) This should explain the lag in the increase of Hb and protein concentration observed in median cubital vein blood But the haemoconcentration ultimately reached for a given compression pressure must be a function of the total filtering area and tissue pressure Since the relatively avascular and tight dermis cannot compete with muscle tissue in this respect, the blood leaving the latter will in the end reach a higher protein concentration

In each tissue ultimately equilibrium is established between the increased intracapillary

pressure on the one hand and the increase in colloid osmotic pressure of the plasma and in tissue pressure on the other and no further increase in protein concentration is observed. This is clearly shown in fig. 23 which was constructed from all the *in vivo* ultra filtration experiments in normal subjects in which samples were taken at regular intervals from 5 to 10 minutes after starting upper arm compression. The percentage increase in protein concentration over the precompression value (concentration in g/Kg H_2O) is plotted against time and for every individual experiment the points are connected by lines.

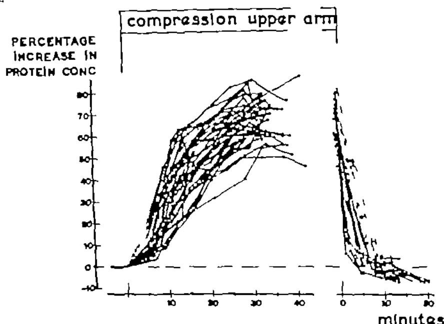


Fig 23 Correlation between duration of compression and increase in protein concentration of venous plasma (sampling site v. cubiti)

After 5 to 8 minutes compression the protein concentration starts to increase rapidly but after about 20 minutes a plateau is reached in most cases. After decompression the protein concentration decreases immediately to drop somewhat below precompression values after about 10 minutes. This is presumably the result of reabsorption of accumulated interstitial fluid.

Fig. 23 demonstrates that in healthy subjects the final increase in protein concentration is considerable. However as will be shown later in patients the equilibrium between intra and extravascular pressure is generally reached at a lower level of protein concentration. This phenomenon is apparently not related to the type of the disease. It might be due to the fact that the normal subjects in our study were all relatively young males, whereas the patients included females and were on the average about 50 years old. In these persons the muscle-skin ratio must have been less favourable for reaching high protein concentrations.

In a previous paragraph we have already mentioned the possibility of protein passing the capillary membrane. If the relative increase in haematocrit and in protein concen-

tration during venous compression are compared it is found that at a compression pressure of 60 mm Hg apparently no or practically no protein leaves the plasma, but that at 80 mm Hg a fair amount must pass the capillary membrane (LANDIS *et al* 1932) In Landis experiments compression was continued for 30 minutes, but similar losses of protein were calculated by GERBRANDY (1951) from 2 experiments in which a compression pressure of 90 mm Hg was applied for 8 minutes For 15 of our experiments we calculated the apparent protein loss in the manner described by Landis. The results indicated without exception a considerable loss of protein in some cases, towards the end of compression the loss of water apparently was accompanied by the loss of 50 / of the protein which it contained in the plasma. However as a discussion of the possible errors in the necessary calculations would lead too far from the purpose of the present study we will restrict ourselves here to the conclusion that during venous compression under the conditions described a considerable loss of protein may occur presumably via the capillary membrane

This in itself does not prevent the use of controlled venous congestion for the study of ion protein interactions provided that all protein fractions leak to an equal degree. In other words the composition of the plasma proteins must remain unchanged during the whole experiment And this appears to be the case. LANDIS *et al* (1932) already could find no difference in the increase of the albumin and of the globulin fractions (determined according to Howe) GERBRANDY *et al* (1960) using paper electrophoresis, found exactly the same percentage increase for albumin as for total protein after 5 to 10 minutes compression of the upper arm at 90–100 mm Hg. Using pressures of 90 mm Hg for 30 to 40 minutes, we (VAN LEEUWEN *et al* 1961) found an equal percentage increase in protein and in respectively ^{131}I labelled albumin cholesterol (which might be considered to represent α and β-globulins) and alkaline phosphatase (which is an α_2-globulin) For the sake of completeness we have again determined the protein composition with paper electrophoresis during two recent *in vivo* ultrafiltration experiments The changes observed were within the error of the determination (table 23) The conclusion is that whatever protein leaves the capillaries has exactly the same composition as plasma protein In view of the difference in molecular size this is an interesting finding At first sight it appears to indicate that the aqueous channels or pores located in the capillary wall (PAPPENHEIMER 1953) must be larger in diameter than the largest β and α-globulin molecules. However as was already mentioned in many subjects a petechial rash develops during compression and as these capillary bleedings must also occur in muscle tissue it is conceivable that most of the leakage of plasma proteins occurs through traumatic rents in the capillary wall This is not in contradiction with the observation that relatively more protein leaves the blood than erythrocytes since the latter will only pass through rather large gaps. A further argument for the traumatic origin of the protein loss can be found in the fact that the latter apparently becomes significant only at pressures higher than 60 mm Hg and increases more rapidly than the volume of ultrafiltrate over the range 60 to 80 mm Hg (LANDIS *et al* 1932)

Changes in O_2 content CO_2 content and pH of the venous forearm blood

As a result of the decrease in blood flow in the presence of constant tissue metabolism one observes a decrease in oxygen content, a decrease in pH and an increase in CO_2 content These are induced by respectively a decrease in P_{O_2} and an increase in P_{CO_2} But

the effect of venous congestion on these parameters is not equally large. Fig. 24 summarises the results of 17 *in vivo* ultrafiltration experiments (4 patients, 10 normal subjects) in which HbO_2, pH and total CO_2 of plasma were determined in a number of samples before, during and after compression of the upper arm.

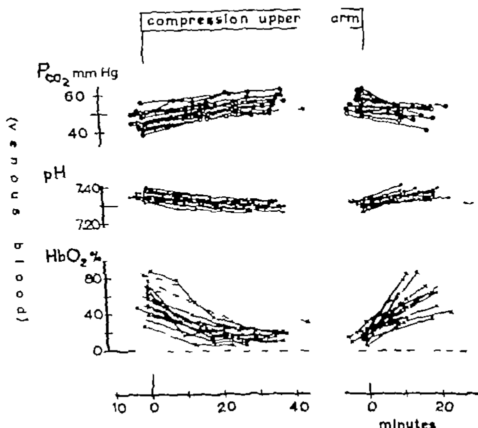


Fig. 24. Correlation between duration of compression and changes in P_{CO_2}, pH and percentage HbO_2 of venous blood (sampling at ... cubiti).

We have refrained from calculating P_{O_2} for the individual experiments because little is known about individual variations in the HbO_2 dissociation curve.

Fig. 24 demonstrates that, for venous P_{CO_2}, pH and HbO_2 alike, the most pronounced changes occur in the first 15 minutes after starting the compression and in the first 10 minutes after decompression. From 20 minutes after the start of compression onwards the values remain fairly constant. Whereas the maximum change in HbO_2 was from 89 to 22 %, the maximum changes in pH and P_{CO_2} were 0.10 pH unit and 14 mm Hg respectively. In fact, the change in pH was so small that we have added fig. 25 in which the trend in pH is drawn on a much larger scale. It was constructed from the 11 *in vivo*

ultrafiltration experiments in normal subjects, in which the pH was determined with every sample It demonstrates better than fig 24 that the main decrease in pH generally occurs in the first 15 minutes of compression

Table 24 summarises the data represented in fig. 24

In addition an average value for P_{O_2} was calculated from the average values for HbO_2 and pH with the aid of the HbO_2 dissociation curve given by DILL (*Handbook of Respiration* 1958).

It would appear that even 10–15 minutes after decompression the values have not yet reached the precompression levels This is in agreement with the observation that after decompression the blood flow remains below the precompression value

Although there is an inverse relationship between the total blood flow through the forearm and the arterial venous (A–V) difference in blood gas content, the latter cannot

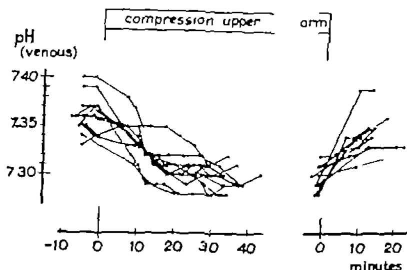


Fig. 25 – Correlation between duration of compression and change in pH of venous blood.

in our experiments be used for calculating the former even if tissue metabolism is kept constant and anaerobic metabolism prevented by completely relaxing the forearm muscles This is due to the fact that the samples were taken from a cubital vein During congestion the relative quantities of blood passing through muscle, through skin and through bone will most certainly alter and thereby cause changes in the composition of cubital vein blood independent of changes in total forearm blood flow It is nevertheless interesting to have some idea of the changes which occur in A–V difference. The more so because we were initially somewhat alarmed by the fact that the change in A–V difference appeared to be definitely larger for O_2 than for CO_2 In two healthy subjects and in one patient with chronic uraemia but with a haemoglobin concentration of 12 g we calculated the A–V differences for O_2 and for CO_2 before, during and after compression (at 90–110 mm Hg) and from these data the apparent respiratory quotient was obtained Fig. 26 shows the result.

The values were obtained in the following manner After the subject had been resting for at least 15 minutes with one arm extended and completely relaxed a needle was introduced in the median cubital vein of the extended arm and a Cournand needle in the brachial artery of the other arm.

A sample of arterial blood was taken at the same time as the first venous sample, thereafter only venous samples were taken. Hb content, HbO_2 %, pH and plasma total CO_2 concentration were determined in all samples. With the aid of the nomogram, which relates plasma CO_2 content to blood CO_2 content (constructed by VAN SLYKE & SENDROY (1928)), blood CO_2 was calculated and expressed in vol %. Blood O_2 content was calculated from Hb concentration and HbO_2 % using the factor 1.34 for converting Hb (g%) in oxygen capacity (vol %). By assuming the composition of the arterial blood constant for the duration of the experiment A–V differences were calculated for every venous sample, and the apparent respiratory quotient (R.Q.) obtained by dividing the CO_2 A–V difference by the O_2 A–V difference.

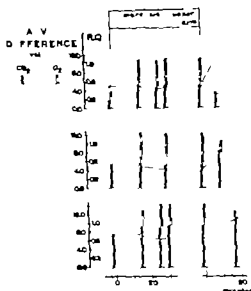


Fig. 26. Correlation between duration of compression and respiratory quotient respectively arterial-venous differences in gas content (venous sampling site vv. cubiti).

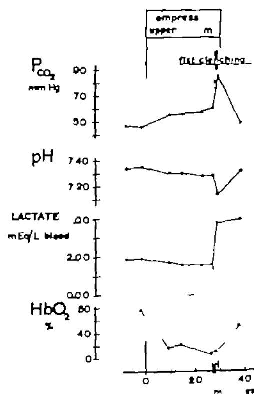
It can be seen that in every instance the same phenomenon was observed. During compression the O_2 A–V difference increases more than the CO_2 A–V difference, but after terminating compression the CO_2 catches up with the O_2. As a result the apparent R.Q. first decreases, but then rises sharply after decompression. In view of the absolute rest of the forearm it is unlikely that changes in tissue metabolism are responsible. In all probability the phenomenon is the result of storage of CO_2 in interstitial and possibly intracellular fluid.

After leaving the tissue cells CO_2 diffuses into the erythrocytes where it is converted into bicarbonate, which in turn diffuses back again into plasma and tissue fluid. In a state of equilibrium the amount of CO_2 carried away by the venous blood is equal to the amount produced by the cells, and the P_{CO_2} of venous blood and tissues remains constant. But when blood flow decreases, only part of the CO_2 produced by the cells is carried away. The P_{CO_2} rises and CO_2 is stored in the forearm tissues. After decompression, when blood flow increases again and end capillary P_{CO_2} drops, the excess tissue CO_2 – mainly present as bicarbonate – diffuses back into the plasma and is removed with the venous blood.

The forearm thus acts in precisely the same way as the body as a whole when its steady state in regard to CO_2 production and transport is disturbed (LILJESTRAND 1916, SHAW 1927, FENN 1928, FARHI & RAHN 1955). A similar storage of O_2 is not possible over the

range of P_{O_2} observed in our experiments as the myoglobin dissociation curve is hyperbolic in shape and situated far to the left on the P_{O_2} scale. As a result a decrease in P_{O_2} from 36 to 15 mm Hg (table 24) would change myoglobin saturation only from about 90 / to about 80 / (MILLIKAN 1939).

The storage function with regard to CO_2 allows the forearm tissues to accomodate a large amount of CO_2 with a relatively small increase in P_{CO_2}. This together with the simultaneous conversion of HbO_2 into the weaker acid Hb probably explains the relatively small decrease in venous pH. Table 25 summarises the decrease of pH after 30 minutes compression as observed in a previous study and in the present study. But the change in pH remains small only if the arm is kept relaxed throughout the experiment. One minute rhythmic clenching of the fist during compression of the upper arm at 85 mm Hg was sufficient to decrease the venous pH from 7.28 to 7.14 and to increase blood lactate from 1.2 mM/L to 2.9 mM/L blood (fig. 27).



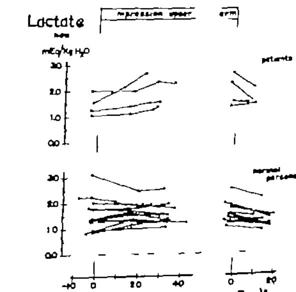


Fig. 28. Effect of compression on lactat concentration of venous plasma (sampling site vv. cubiti)

Fig. 27. Effect of muscle contraction on composition of venous blood from forearm (sampling site mediana cubiti).

Lactate formation

Complete relaxation is difficult and it might therefore be asked whether during compression no anaerobic metabolism occurs resulting in an increase in lactic acid content of the venous blood. In three healthy persons comparison of lactic acid A–V difference with O_2 A–V difference before, during and after compression for periods up to 35 minutes indicated that no significant increase in lactic acid formation occurred under the conditions of our experiment (Table 3 in VAN LEEUWEN *et al.*, 1961). Furthermore, in 17 experiments (10 normal subjects and 4 patients) venous blood lactate was determined before, during and after compression of the upper arm. The results are shown in fig. 28.

It should be noted that the values are given in mEq per Kg H_2O. They were calculated from blood values with the aid of the equation H_2O (g per litre blood) = 996 — 0.82 (Hb + plasma protein (both in g per litre blood)), where the constants are the same as in the equation which we derived from plasma water determination in the ultrafiltration experiments with normal subjects (chapter VI).

In the *normal subjects* when the lactate concentration was low to begin with a slight rise was seen during venous compression but when it was high before compression a slight decrease was observed. After decompression a – small – decrease occurred in all cases. Table 26 gives the average values obtained in the 13 experiments. We have no explanation for the one experiment in which the value remained well above 2.00 mEq/Kg H_2O. As far as could be ascertained the subject had not unduly exercised himself before the start of the experiment. However as our building contains no elevator and the experiments were done on the second floor we cannot exclude the possibility that the subject scaled the stairs in a hurry in his eagerness to participate in the experiment.

At any rate, from the fact that both mean value and range are larger before compression than after it must be concluded that the normal subjects had not rested sufficiently long before the experiment started. But the changes in concentration are small enough to support the conclusion that lactate formation must have remained negligible in the normal subjects.

In the four *patients* studied the changes were somewhat larger and the one patient who had anaemia (Hb 9 g %) showed a definite increase during compression. In his case HbO dropped to 7 % and anaerobic metabolism appears to have occurred.

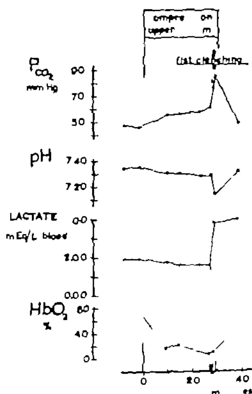
As it was found practically impossible to determine lactate in all *in vivo* ultrafiltration experiments because of the rather laborious technique, we have assumed that in the case of the normal subjects and of the patients with a normal Hb concentration the lactate concentration of the venous blood remained constant. In the few patients with anaemia who were studied, an increase may have occurred during compression.

Changes in the concentration of inorganic phosphate

No attempt has been made to determine sulphate or organic anions other than lactate. But inorganic phosphate was studied for several reasons. Quantitatively it is the fourth anion after Cl, HCO_3 and protein; it is a representative of intracellular anions and it is reported to be bound by plasma proteins. The latter conclusion was reached by GREENE & POWER (1931) on the strength of the concentration ratio observed in their *in vivo* dialysis experiments, and from their results it would appear that from 10 to 20 % of the inorganic phosphate present in plasma behaves as if it was complex-bound by plasma protein. WALSER (1960), using the ultrafiltration technique of Toribara, calculated that in normal plasma about 25 % of inorganic phosphate must be protein-bound. In plasma of 22 hyperphosphataemic uraemic patients (two of whom had a phosphate concentration higher than 5 mM/L) this fraction was on the average 14 %. But these values are probably too high because of an error in technique. In a more recent publication WALSER (1961) reports that on the average 13 % of inorganic phosphate is protein-bound in normal persons. The results of SEEKLES (1936), who studied bovine serum by means of ultrafiltration, show no evidence of binding and the observations of HOPKINS *et al* (1952) appear to indicate that in normal plasma not more than 10 % of inorganic phosphate is bound by the plasma protein.

range of P_{O_2} observed in our experiments, as the myoglobin dissociation curve is hyperbolic in shape and situated far to the left on the P_{O_2} scale. As a result a decrease in P_{O_2} from 36 to 15 mm Hg (table 24) would change myoglobin saturation only from about 90 % to about 80 % (MILLIKAN 1939).

The storage function with regard to CO_2 allows the forearm tissues to accomodate a large amount of CO_2 with a relatively small increase in P_{CO_2}. This together with the simultaneous conversion of HbO_2 into the weaker acid Hb probably explains the relatively small decrease in venous pH. Table 25 summarises the decrease of pH after 30 minutes compression as observed in a previous study and in the present study. But the change in pH remains small only if the arm is kept relaxed throughout the experiment. One minute rhythmic clenching of the fist during compression of the upper arm at 85 mm Hg was sufficient to decrease the venous pH from 7.28 to 7.14 and to increase blood lactate from 1.2 mM/L to 2.9 mM/L blood (fig. 27).



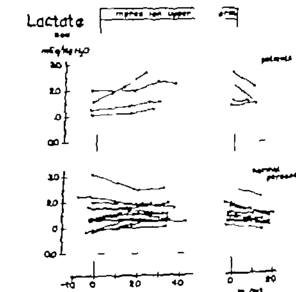


Fig. 28. Effect of compression on lactate concentration of venous plasma (sampling site ... vv. cubiti)

Fig. 27. Effect of muscle contraction on composition of venous blood from forearm (sampling site ... mediana cubiti)

Lactate formation

Complete relaxation is difficult and it might therefore be asked whether during compression no anaerobic metabolism occurs resulting in an increase in lactic acid content of the venous blood. In three healthy persons comparison of lactic acid A–V difference with O_2 A–V difference before, during and after compression for periods up to 35 minutes indicated that no significant increase in lactic acid formation occurred under the conditions of our experiment (Table 3 in VAN LEEUWEN *et al.*, 1961). Furthermore in 17 experiments (10 normal subjects and 4 patients) venous blood lactate was determined before, during and after compression of the upper arm. The results are shown in fig. 28.

The fact that the overall increase in plasma anions is much smaller than the increase in total blood CO_2 (both expressed per Kg H_2O) is explained by the effect of the conversion of HbO_2 into Hb. As a result of the decrease in valencies of the (non-filtrable) haemoglobin molecules the Donnan ratio for the erythrocyte-membrane increases and consequently the sum concentration of Cl and HCO_3 increases relatively more in the erythrocytes than in the plasma (VAN SLYKE *et al.* 1923; HENDERSON 1931). Moreover, the desaturation of Hb leads to an increase in the amount of CO_2 bound as carbamino-haemoglobin (FERGUSON & ROUGHTON, 1934 a, b).

As the CO_2 dissociation curve of blood is practically linear over the range 40 to 60 mm Hg P_{CO_2}, we can use Dill's data for estimating the changes to be expected during our experiments. Table 24 shows that in our experiments the P_{CO_2} of venous blood increased on the average from 46 mm Hg to 54 mm Hg, *i.e.* 8 mm Hg. On the strength of Dill's values one might therefore expect that during venous compression the pH decreases 0.04, plasma HCO_3 increases 2.5 mEq, plasma Cl decreases 2.1 mEq and the sum of Cl and HCO_3 increases 0.4 mEq, all per Kg H_2O. Actually, averaging our 34 observations on normal subjects, the changes observed were: for pH −0.04; for HCO_3 +1.3 mEq; for Cl −1.3 mEq, and for Cl+HCO_3 0.0 mEq per Kg H_2O.

The decrease in pH appears correct, but the changes in HCO_3 and Cl are only half those expected. Even if we acknowledge the fact that Dill *et al.* obtained their values for venous blood from calculations based on *in vitro* experiments, the small increase in plasma HCO_3 in comparison with the decrease in pH observed in our experiments needs an explanation. Especially so since the decrease in Cl was quantitatively in good agreement with the increase in HCO_3.

The discrepancy between pH decrease and HCO_3 increase might be due to an error in pH measurement as a result of temperature changes in venous blood during compression, but experimental evidence (see later) indicates that the average effect of this error on the measured difference in pre- and endcompression pH must have been negligible. The discrepancy is therefore presumably a real one and it suggests the addition to the blood of another acid beside H_2CO_3. It is unlikely that this is an organic acid (in the ordinary sense of the word) since we could detect no significant increase in venous lactate concentration. But an explanation might be provided by the effect of an increase in Hb concentration on the CO_2 absorption curve of blood (VAN SLYKE *et al.* 1923). Above their isoelectric point haemoglobin and plasma proteins act as acids, with which bicarbonate competes for H-ions. As a consequence, in CO_2 absorption curves of blood with normal plasma and cell composition but with increasing haemoglobin concentration, a decrease of bicarbonate concentration per Kg blood should be observed at pH ± 7.30. *In vitro* experiments on oxygenated blood of a Mongolian pony confirmed these theoretical considerations (VAN SLYKE *et al.* 1923). In our experiments with normal persons Hb concentration increased on the average 30 %, whereas in Dill's calculation Hb concentration was considered to remain constant. It is tempting to estimate with the aid of Van Slyke's data whether the increase in Hb observed in our experiments could have the effect of halving the increase in plasma HCO_3 to be expected from the observed decrease in pH.

To use data obtained on the blood of a Mongolian pony in this manner is a hazardous enterprise. Van Slyke *et al.* stress the fact that the absolute values given may differ considerably for other blood samples and that their figures must be considered to indicate merely the relative magnitude of the values expected. However, DAUTREBANDE *et al.* (1923) observed a 10 % decrease in the CO_2

In fig. 29 our observations during *in vivo* ultrafiltration are shown. We studied 4 normal persons (the lower values), 1 patient with osteolytic metastases (the median values) and 3 patients with chronic uraemia (the highest values). Only in the case of the highest concentration was a significant change observed and this was a decrease limited to the last sample during compression and the one sample taken after decompression. As a slight decrease in phosphate concentration was to be expected as a result of the change in Donnan equilibrium (to be discussed in a following section) our findings are consistent with the existence of a small amount of protein-bound inorganic phosphate. Its exact magnitude cannot be calculated without knowledge of the magnitude of the change in Donnan ratio but from our results it would appear that it cannot be more than 5 % of the inorganic phosphate concentration in normal plasma.

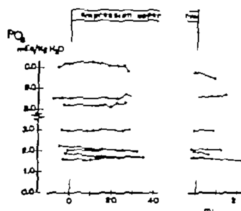


Fig. 29. Effect of compression on concentration of inorganic phosphate in venous plasma (sampling at ... cubiti).

Changes in the distribution of ions between plasma and erythrocytes – Their effect on plasma Cl and HCO_3 concentration

During venous congestion the uptake of CO_2 per unit volume blood increases. After passing into the erythrocytes the CO_2 is converted into H_2CO_3 and this in turn becomes HCO_3 since the H ion concentration in the erythrocytes decreases as a result of the conversion of the stronger acid HbO_2 into the weaker acid Hb.

The increase in osmolality resulting from the increase in intracellular HCO_3 concentration causes the erythrocytes to attract some water. But at the same time HCO_3 leaves the cells for the plasma and Cl moves from the plasma into the erythrocytes, until the concentration ratios for HCO_3 and Cl have regained their normal relation.

These now well known processes have been elucidated by Hamburger, by Henderson, Dill, Bock and other associates, by Van Slyke and his group and by Roughton and associates; the reader is referred to VAN SLYKE *et al.* (1923), HENDERSON (1931), DILL *et al.* (1937[1, 2]) and ROUGHTON (1954) for qualitative and quantitative data.

For our present problem the difference in composition of arterial and venous blood in resting men found by DILL *et al.* (1937[1]) is of special interest. From their data it can be calculated that in physiological conditions an increase of 6.5 mm Hg in P_{CO_2} of the blood is accompanied by a decrease of 0.03 in pH. Simultaneously HCO_3 content of the serum increases by 1.97 mEq per kg H_2O while Cl content decreases by 1.67 mEq per Kg H_2O.

equilibrium, the increasing protein concentration of plasma will force the filtrable anions in plasma to decrease in concentration. Whereas the total concentration of the filtrable cations increases, the concentration of cation not associated with protein must decrease parallel with that of the filtrable anions. The magnitude of this effect depends on the Donnan ratio prevailing at the normal protein concentration of plasma and it must affect our calculation of protein-bound cation. This is demonstrated in fig. 30, where the

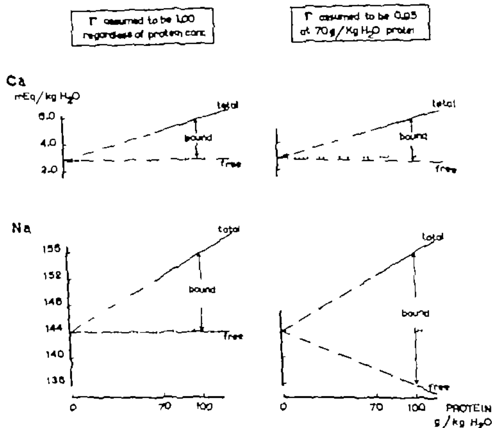


Fig. 30. Effect of the existence of the Donnan-equilibrium on the calculation of the protein-bound and the free fraction of Ca and Na. See text.

Ca and Na regression lines from the experiment depicted in fig. 19 and fig. 20 are reproduced. On the left hand side fig. 30 demonstrates how the *amount of cation* bound per 100 g of protein is actually calculated, i.e. on the assumption that the concentration of 'free' filtrable cation remains constant. In other words, as if the Donnan ratio were 1.000 regardless of the protein concentration in plasma. This picture is clearly erroneous. In reality the concentration of 'free' filtrable cation in plasma decreases with increasing protein concentration as shown in fig. 30 on the right-hand side. As a result the (total) plasma concentration of a cation *increases less than* the concentration of its protein bound fraction. We can make an estimate of this effect.

combining power of venous forearm blood after 25–40 minutes of stasis, induced by compressing the upper arm at 100–125 mm Hg. In the two experiments shown in their table II the Hb concentration increased by 25 ‰ and in both lactic acid concent of the blood remained low and constant.

The conclusions of the authors concerning the *in vivo changes during their experiments* are difficult to follow because pH was calculated from the CO_2 content of alveolar air and the total CO_2 of venous blood, using *in vitro determinations of the CO_2 absorption curve. But since the* latter was determined for both pre and endcompression samples by equilibrating blood at 37°C against gas of known CO_2 content, they can be *compared. They show that at a pH of about* 7.30 the CO_2 combining power of the blood decreased from 23 to 20 mM/L with a concomitant increase in Hb from about 80 to 105 ‰. *This change is of the same magnitude as that given by* VAN SLYKE *et al* (1923) in their fig. 7 and it suggests that the latter can be used for a quantitative estimate of the effect of Hb concentration on plasma HCO_3 in our experiments.

This was done in the following manner after recalculating the HCO_3 concentrations in fig. 7 of VAN SLYKE *et al* (1923) from kg blood to litre blood by using data on the relation between Hb concentration and specific gravity of blood (VAN SLYKE *et al* 1950), one can calculate with the aid of VAN SLYKE and SENDROY s (1928) nomogram (relating plasma CO_2 content to blood CO_2 content at varying Hb concentration and oxygen *saturation) the effect of increasing Hb on plasma bicarbonate concentration Thus,* one can estimate the change in HCO_3 concentration per litre plasma, to be expected when pH decreases from 7 35 to 7 30 and HbO_2 from 60 / to 20 / (very nearly the conditions in our experiments) both for the case that Hb concentration remains constant at 15 g and for the case that Hb concentration changes from 15 g / to 20 g ‰. In the first instance – *i e the condition assumed by Dill – plasma HCO_3 should increase from* 23 8 mM/L to 25 2 mM/L, *i e* 1 4 mM/L. In the second case – our conditions – the change should be from 23 8 mM/L to 24 5 mM/L, *i e* only 0 7 mM/L.

It is not suggested that the absolute values thus obtained are accurate, but the result of the calculation indicates that the increase in Hb concentration can explain the apparent discrepancy between the magnitudes of the pH decrease and the HCO_3 increase observed in our experiments.

It might be asked whether so much reasoning about concentration differences that are after all not larger than about 1 0 mEq is not somewhat ridiculous. The answer must be that it is not. By using the forearm as an apparatus for ultrafiltration we obviously change more factors than those which we aim to use in our final calculation. If no attempt is made to understand *all factors* and to assess them in at least a roughly quantitative way our final conclusions will remain *questionable to a high degree*

Apart from the changes in HCO_3 and in Cl it is important to consider *the change in* their sum concentration As mentioned already in the experiments with normal subjects we found no change during venous compression whereas the sum concentration should *have increased about 0.2 mEq as a* result of the increased uptake of CO_2 The difference is a small one In fact an astonishingly small one considering the effect which one would expect from the change in *Donnan equilibrium between plasma and interstitial* fluid This will be discussed now

Changes in the distribution of ions between plasma and interstitial fluid – The effect of the increase in protein concentration on the Donnan equilibrium

If we assume *that the protein* concentration of the interstitial fluid is negligible and remains so during venous compression then, according to the dictates of the Donnan

Another assumption w made was that the protein concentration of interstitial fluid remained zero throughout. Earlier investigations appeared to indicate that it was 5 g/L at the highest, but DRINKER and others maintained from their study of the protein concentration of lymph that it could be considerably higher. A review of the literature up to 1934 was given by PETERS (1935), who concluded that Drinker's values were too high. But more recent work favours the concept of a fairly large turnover of proteins between blood and lymph in interstitial fluid and appears to put Drinker in the right (MANERY 1954). From their experiments on fluid transport across the capillary membrane PAPPENHEIMER & SOTO-RIVERA (1948) concluded that the interstitial fluid in skeletal muscle contains bout 7 g/L protein. But GEBBRANDY (1951) correctly pointed out that in the balance of forces influencing water transport across the capillary membrane the influence of tissue pressure itself was not sufficiently taken into consideration. Consequently the authors underestimated the colloid osmotic effect of interstitial fluid protein and thus protein concentration.

A further point to be ealised is that our compression pressures are well above the pressure at which LANDIS *et al.* (1932) found that protein started to leave the blood vessels. Using their method of comparing changes in haematocrit and in total protein, w calculated that consider able amount of plasma protein leaves the blood in the forearm during compression t 100 mg Hg. This suggests that in our experiments the increase in the difference between non-filtrable valencies present on both sides of the capillary membrane is definitely less than will appear from the increase in the intravascular protein concentration alone.

We must conclude that the magnitude of the effect due to changes in Donnan ratio during venous compression remains an open question. In calculating the protein-bound fractions of Na, Ca and Mg probably a certain error will remain. From the fact that we observed no change in the sum concentration of Cl + HCO_3 during compression, and assuming 2 mEq of Cl to be complex bound by 70 g normal plasma protein, one would guess that for Na the underestimate will be of the order of 3–4 mEq per 100 g of protein and for Ca about 0 1–0.2 mEq per 100 g protein.

The calculation of NCE is clearly not affected by these changes in Donnan equilibrium since the latter affects the 'free filtrable cations to the same extent as the 'free filtrable anions

Changes in cation concentration not caused by the increase in protein concentration

There is no reason to suppose that the 'free concentration of Ca and Mg changed during our experiments, as their plasma concentrations before and after compression were the same when considered in relation to protein concentration. But it was not always so for Na and practically never so for K

( ) *Potassium* In earlier experiments (GEBBRANDY *et al.*, 1960) it was found that K increased relatively more than Na during venous compression and that its concentration in contrast to that of the other cations did not return to precompression values after compression was released There is therefore an increase in 'free K in plasma during enous compression The cause i not clear Although apparently *in the case of normal* erythrocytes no K lea es the cell during increased uptake of CO_2 from and delivery of O_2 to the tissues (V N SLYKE *et al* 1923 TOSTESON *et al* 1952) it must be doubted whether previous K determinations ha e been accurate enough to detect a systematic increase of the magnitude observed in ou experiments. It is also possible that the excess K stems from the tissue cells, in response to the slight acidaemia brought about by the transersion of CO into H_2CO_3. The fact that the K concentration remains higher after

If plasma contained no protein the concentration of the cations would be equal on either side of the capillary membrane. As soon as protein is added, filtrable cation and anion move from plasma to interstitial fluid until their concentration ratios across the capillary membrane equal the Donnan ratio for the given protein concentration. The quantity of filtrable ion that must leave the plasma to effect this change in concentration ratio depends on the relative volumes of plasma and interstitial fluid in the forearm. If we assume the latter to be thrice as large as the former (and consider the forearm during venous compression a closed system) the diffusion of a given amount of cation into the interstitial space will decrease plasma concentration thrice as much as it will increase the concentration in interstitial fluid. If we further assume the Donnan ratio to be 0.95 at a protein concentration of 70 g/Kg H_2O (see chapter II) we find that, while protein increases, free cation and anion decrease until at a protein concentration of 100 g/Kg H_2O the concentration of "free" filtrable (univalent) ions in plasma has become 0.947 times their concentration at zero protein. At the same time the concentrations in interstitial fluid should have increased to 1.018 times the initial concentration. Since for the bivalent ions the square of the Donnan ratio must be used, the relative decrease in plasma concentration is more in their case.

For the particular example in fig. 30 we can thus calculate that neglecting the Donnan effect will make us underestimate protein bound Na by 7.6 mEq and protein-bound Ca by 0.30 mEq, both per 100 g of protein.

In reality, however, the Donnan effect must have been smaller under the conditions of our experiments. If fig. 30 (right hand side) were a quantitatively correct representation of the Donnan effect in our experiments, then during compression the sum concentration of Cl and HCO_3 (which was on the average 140 mEq/Kg H_2O) should have decreased by about 7.2 mEq for every 100 g increase in protein concentration. As the average increase in protein concentration in the 34 experiments with normal persons was 48 g we must expect the actual decrease to have been 3.5 mEq/Kg H_2O. Since the extra CO_2 uptake should have increased the sum concentration of Cl and HCO_3 by 0.2 mEq/Kg H_2O, we arrive at the conclusion that according to fig. 30 the overall effect of haemoconcentraction on the sum concentration of Cl + HCO_3 should have been a decrease of 3.3 mEq/Kg H_2O. Instead it was found to be zero.

This discrepancy is only partially explained by the fact that some chloride appears to be bound by albumin at physiological pH. Even if this is also true for conditions *in vivo* not more than 3 mEq of Cl should be complex bound by the amount of albumin present in 100 g normal plasma protein (see chapter I) and the observed 48 g increase in total protein concentration would therefore counteract the influence of the Donnan equilibrium by increasing Cl concentration with 1.5 mEq/Kg H_2O at the most. This still leaves an expected decrease in Cl + HCO_3 of 1.5 to 2.0 mEq/Kg H_2O. It would therefore appear that the Donnan effect during venous compression is smaller than that calculated on the assumption of a Donnan ratio 0.95 for normal plasma. In fact if the above reasoning is correct the Donnan ratio must be somewhere between 0.97 and 0.98 when the protein concentration is 70 g/Kg H_2O. In view of the data discussed in chapter II this seems rather high. But other explanations are also possible.

In the construction of fig. 30 we assumed the interstitial fluid with which the blood in the forearm is in equilibrium to be considerably larger in volume than the plasma. But recent work reviewed by MANERY (1954) indicates that especially in skeletal muscle the amount of interstitial fluid must be so small that only a thin film will envelop the blood vessels, connective tissue fibres and muscle fibres, and our assumption concerning the relative proportions of plasma and interstitial fluid volume in the forearm must be wrong. This means that for a given change in the Donnan ratio the decrease in the plasma concentration of "free" filtrable ions will be less, while the increase in their interstitial concentration will be more than was calculated above.

Another assumption we made was that the protein concentration of interstitial fluid remained zero throughout. Earlier investigations appeared to indicate that it was 5 g/L at the highest, but DRINKER and others maintained from their study of the protein concentration of lymph that it could be considerably higher. A review of the literature up to 1934 was given by PETERS (1935), who concluded that Drinker's values were too high. But more recent work favours the concept of a fairly large turnover of proteins between blood and lymph via interstitial fluid and appears to put Drinker in the right (MANERY 1954). From their experiments on fluid transport across the capillary membrane PAPPENHEIMER & SOTO-RIVERA (1948) concluded that the interstitial fluid in skeletal muscle contains about 7 g/L protein. But GERBRANDY (1951) correctly pointed out that in the balance of forces influencing water transport across the capillary membrane the influence of tissue pressure itself was not sufficiently taken into consideration. Consequently the authors underestimated the colloid osmotic effect of interstitial fluid protein and thus protein concentration.

A further point to be realised is that our compression pressures are well above the pressure at which LANDIS *et al.* (1932) found that protein started to leave the blood vessels. Using their method of comparing changes in haematocrit and in total protein, we calculated that a considerable amount of plasma protein leaves the blood in the forearm during compression at 100 mg Hg. This suggests that in our experiments the increase in the difference between non-filtrable valencies present on both sides of the capillary membrane is definitely less than will appear from the increase in the intravascular protein concentration alone.

We must conclude that the magnitude of the effect due to changes in Donnan ratio during venous compression remains an open question. In calculating the protein-bound fractions of Na, Ca and Mg probably a certain error will remain. From the fact that we observed no change in the sum concentration of Cl + HCO_3 during compression, and assuming 2 mEq of Cl to be complex-bound by 70 g normal plasma protein, one would guess that for Na the underestimate will be of the order of 3–4 mEq per 100 g of protein and for Ca about 0.1–0.2 mEq per 100 g protein.

The calculation of NCE is clearly not affected by these changes in Donnan equilibrium since the latter affects the 'free' filtrable cations to the same extent as the 'free' filtrable anions.

Changes in cation concentration not caused by the increase in protein concentration

There is no reason to suppose that the 'free' concentration of Ca and Mg changed during our experiments, as their plasma concentrations before and after compression were the same when considered in relation to protein concentration. But it was not always so for Na and practically never so for K.

(a) *Potassium*. In earlier experiments (GERBRANDY *et al.* 1960) it was found that K increased relatively more than Na during venous compression and that its concentration – in contrast to that of the other cations – did not return to precompression values after compression was released. There is therefore an increase in 'free' K in plasma during venous compression. The cause is not clear. Although apparently in the case of normal erythrocytes no K leaves the cell during increased uptake of CO_2 from and delivery of O_2 to the tissues (VAN SLYKE *et al.* 1923, TORTESON *et al.* 1952) it must be doubted whether previous K determinations have been accurate enough to detect a systematic increase of the magnitude observed in our experiments. It is also possible that the excess K stems from the tissue cells, in response to the slight acidaemia brought about by the transversion of CO_2 into H_2CO_3. The fact that the K concentration remains higher after

If plasma contained no protein the concentration of the cations would be equal on either side of the capillary membrane. As soon as protein is added, filtrable cation and anion move from plasma to interstitial fluid until their concentration ratios across the capillary membrane equal the Donnan ratio for the given protein concentration. The quantity of filtrable ion that must leave the plasma to effect this change in concentration ratio depends on the relative volumes of plasma and interstitial fluid in the forearm. If we assume the latter to be thrice as large as the former (and consider the forearm during venous compression a closed system") the diffusion of a given amount of cation into the interstitial space will decrease plasma concentration thrice as much as it will increase the concentration in interstitial fluid. If we further assume the Donnan ratio to be 0.95 at a protein concentration of 70 g/Kg H_2O (see chapter II) we find that, while protein increases, free cation and anion decrease until at a protein concentration of 100 g/Kg H_2O the concentration of 'free' filtrable (univalent) ions in plasma has become 0.947 times their concentration at zero protein. At the same time the concentrations in interstitial fluid should have increased to 1.018 times the initial concentration. Since for the bivalent ions the square of the Donnan ratio must be used, the relative decrease in plasma concentration is more in their case.

For the particular example in fig. 30 we can thus calculate that neglecting the Donnan effect will make us underestimate protein-bound Na by 7.6 mEq and protein bound Ca by 0.30 mEq, both per 100 g of protein.

In reality, however, the Donnan effect must have been smaller under the conditions of our experiments. If fig. 30 (right hand side) were a quantitatively correct representation of the Donnan effect in our experiments, then during compression the sum concentration of Cl and HCO_3 (which was on the average 140 mEq/Kg H_2O) should have decreased by about 7.2 mEq for every 100 g increase in protein concentration. As the average increase in protein concentration in the 34 experiments with normal persons was 48 g we must expect the actual decrease to have been 3.5 mEq/Kg H_2O. Since the extra CO_2 uptake should have increased the sum concentration of Cl and HCO_3 by 0.2 mEq/Kg H_2O we arrive at the conclusion that according to fig. 30 the overall effect of haemoconcentraction on the sum concentration of Cl + HCO_3 should have been a decrease of 3.3 mEq/Kg H_2O. Instead it was found to be zero.

This discrepancy is only partially explained by the fact that some chloride appears to be bound by albumin at physiological pH. Even if this is also true for conditions *in vivo* not more than 3 mEq of Cl should be complex bound by the amount of albumin present in 100 g normal plasma protein (see chapter I) and the observed 48 g increase in total protein concentration would therefore counteract the influence of the Donnan equilibrium by increasing Cl concentration with 1.5 mEq/Kg H_2O at the most. This still leaves an expected decrease in Cl + HCO_3 of 1.5 to 2.0 mEq/Kg H_2O. It would therefore appear that the Donnan effect during venous compression is smaller than that calculated on the assumption of a Donnan ratio 0.95 for normal plasma. In fact if the above reasoning is correct the Donnan ratio must be somewhere between 0.97 and 0.98 when the protein concentration is 70 g/Kg H_2O. In view of the data discussed in chapter II this seems rather high. But other explanations are also possible.

In the construction of fig. 30 we assumed the interstitial fluid with which the blood in the forearm is in equilibrium to be considerably larger in volume than the plasma. But recent work reviewed by MANERY (1954) indicates that especially in skeletal muscle the amount of interstitial fluid must be so small that only a thin film will envelop the blood vessels, connective tissue fibres and muscle fibres, and our assumption concerning the relative proportions of plasma and interstitial fluid volume in the forearm must be wrong. This means that for a given change in the Donnan ratio the decrease in the plasma concentration of 'free' filtrable ions will be less, while the increase in their interstitial concentration will be more than was calculated above.

MOOK 1931). The reported rates are sufficiently high to guarantee a condition of equilibrium across the capillary wall during the time necessary for taking a sample.

Effect of venous congestion on the temperature of venous forearm blood – Consequences for the measurement of blood pH

Since inactive muscles produce practically no heat, the temperature of a resting limb depends on the heat apported by the arterial blood and on its insulation. Even when surrounded by air and when no sweating occurs the latter is not sufficient to prevent a decrease in temperature of the blood during its passage through the limb (HARRIS & MARVIN 1927–1929, ALDENHOVEN & KORTH 1932, HORVATH *et al.* 1950, EICHNA *et al.* 1951). The blood circulating through skin areas will cool more than the blood flowing through the muscles. As a result the difference in temperature measured between arterial and venous blood must vary according to the area from which the venous blood is drawn. It should be largest in the superficial veins draining the back of the hand. This is indeed so. With copper-constantan thermoelements (soldered into a needle-tip, or introduced into a plastic catheter) the following intravenous temperatures were measured in man (resting and at 20°–24°C ambient temperature):

Dorsal hand vein	34.3–35.8°C	(HARRIS & MARVIN 1927–'29)
Forearm vein (cubital?)	35.3–36.6°C	(ALDENHOVEN & KORTH 1932)
Vena subclavia	36.0°–36.7°C	(EICHNA *et al.* 1951, if we assume rectal temperature to have been 37.0°C in their subjects).

Furthermore LIPPROSS & SCHEGA (1941) found on one occasion a temperature difference of 1.6 C between art. radialis and vena mediana cubiti.

It follows that the temperature of the blood sampled from the cubital veins during our experiments must have been 1.5 to 2.0 degrees lower than 37.5°C, *i.e.* the temperature at which all our pH measurements were made. As a consequence our pH values are somewhat too low, since measuring pH of whole blood at a temperature higher than the actual temperature of the blood results in a pH decrease of 0.014 to 0.015 unit for every degree Celsius (ROSENTHAL 1948, SIGGAARD ANDERSEN 1963). We found the same relation. But as long as the temperature of the venous blood remains constant during an *in vivo* ultrafiltration experiment, the resulting error in pH measurement will not influence the calculation of NCE. However, compression of the upperarm considerably decreases blood flow through the forearm. It is therefore to be expected that the temperature of the venous blood decreases during prolonged compression. Indeed, ALDENHOVEN & KORTH (1932) in fig. 3 of their article show the effect of 3 minutes compression of the upperarm on the temperature in the forearm vein: a decrease from 36.0° to 34.8°C was observed, although the forearm was covered by a cloth. If a similar decrease in venous blood temperature takes place in our experiments, the error made by measuring all samples at 37.5°C will increase during compression, and thus suggest a larger change in pH than actually occurs. For instance, if no real change in pH occurs but the temperature drops 2°C, the pH will seemingly decrease by 0.03. As this is about the pH change measured during compression, it was necessary to obtain data on the temperature of the blood as sampled in our experiments.

The temperature was measured intravenously by means of a miniature NTC resistance (Philips B 8320 02P/1K) sealed into the tip of a closed polyethylene catheter (external diam. 1 mm).

decompression could be explained by the lag in the removal of HCO_3 stored in the tissues during venous compression

This kind of hysteresis phenomenon makes it necessary to consider already here the differences in the overall balance of cations and anions before and after compression. From the 34 observations in normal persons we calculated the mean difference for the sum concentration of cations, for Na, for K, for Cl + HCO_3, for protein and for pH Furthermore the difference in lactate concentration observed in the 13 experiments in normal subjects (table 26) was assumed to be representative for all 34 experiments. The result is shown in table 27

(Cat min Cl + HCO_3 + lactate) decreased by 0 25 mEq/Kg H_2O pH decreased by 0 01 and there was no change in protein concentration Assuming the buffer value of plasma protein to be 10 mEq per 100 g protein and per pH unit change (see chapter II), we are left with a difference of 0 15 mEq between pre and postcompression values which is not accounted for In view of the cumulative errors involved this may be considered to be non-significant and the result therefore indicates that on the average the NCE before and after venous compression is unchanged From table 27 it can also be seen that the average change in the cations is sol ly due to the change in K.

(b) *Sodium* The ingestion of water and of Na and Cl does not occur in a fixed ratio and the adjustment of plasma osmolality is not instantaneous. Therefore occasional changes in the Na (and Cl) concentration of plasma are to be expected which are independent of protein concentration In our experiments the changes have been minimised by doing all investigations in the morning, allowing at the most 150 ml tea to be taken not later than one hour before the start of the experiment. Only in 8 out of the 34 experiments with normal subjects was a difference of 0 5 mEq/Kg H_2O or more observed between the Na concentration of the pre and that of the postcompression samples, the largest difference being 1 3 mEq/Kg H_2O The changes were in both directions and the mean difference between pre and postcompression samples for the whole group of normal subjects was 0 0 mEq/Kg H_2O (see table 27)

In the 8 cases mentioned the calculation of protein-bound Na will be somewhat in error since one cannot be certain that the change in osmolality was a gradual one But the calculation of NCE should not be affected since the effect on Na and Cl concentration must have been in the same direction

In the observations on patients we found the same relative constancy in pre and post compression Na values, but owing to the fact that in some patients no postcompression samples were obtained no average change was calculated for this group

The time necessary for the filtrable ions to establish a new equilibrium between plasma and interstitial fluid, and between plasma and erythrocytes

Pappenheimer (1953) concluded on rather firm grounds that for the small ions the passage across the capillary wall occurs at a rate which is about 50 times higher than the rate at which the ions are apported with the blood In this respect no difficulties are to be expected during venous compression

The rate of CO_2 uptake by and H_2CO_3 formation in the erythrocytes appears to be of the order of tenths of seconds, the rate of the Cl shift of the order of seconds (Dirken &

pected, the temperature is in the first 10 minutes of compression higher than in the pre and in the postcompression period.

The fact that the change in temperature differs according to the place of sampling may be explained in the following manner During compression venous blood will start to accumulate in the forearm. Since venous blood is colder than arterial blood but warmer than the surrounding tissues, the temperature in the areas surrounding the main arteries will at first remain unaltered or decrease slightly whereas in the areas away from the main arteries the temperature will at first increase. When the distension of the venous

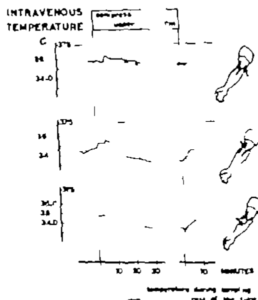


Fig 31 Effect of compression on temperature of blood in different cubital veins. See text.

reservoir has come to an end and compression still continues, the overall loss of heat manifests itself everywhere by a decrease in temperature. The latter will be most pronounced in the areas containing no main artery

An estimate of the temperature changes that must have occurred during our experiments remains speculative for various reasons. The number of experiments was very small and limited to one person. It is to be expected that the changes will be quantitatively different in different arms. Furthermore, although in most of our experiments the blood was sampled from the ulnar cubital ein, in a number of cases the median cubital vein was chosen for technical reasons. Generally this was not mentioned in the protocol and we are therefore unable to correlate the pH decrease observed in the individual cases with the sampling site. However from the l terature and from our own experiments the following conclusions seem justified

( ) On the verage the temperature of the blood at the moment of sampling must have been 1 5 to 2.5°C lower than 37.5°C. Our pH values are therefore on the average 0 03 too low At the total CO_2 concentrations observed in our experiments this causes plasma HCO_3 t be underestimated by 0.1 mEq/Kg H_2O

The NTC resistance (1000 Ω) was connected to a recording potentiometer (Variam G10) via a bridge of Wheatstone. At the voltage used (5 V) no heat generation could be detected. The 90 response time of NTC recording system was less than 1" for a temperature change of 2°C the sensitivity was such that 0 1 C corresponded with 1.3 mm deflection over the temperature range 32 to 37 C. The NTC resistance did not respond to mechanical stimuli or changes in the flow of the surrounding fluid (0–20 L/min). The catheter NTC combination could be sterilised by boiling for 20 minutes without changing its temperature-resistance characteristic. The calibration was done immediately after concluding an experiment at several temperatures between 3 and 37°C against the thermometer used in the pH meter circuit (smallest division 0.01 C). The drift was less than 2 mm deflection per hour

During compression a change in the relative contribution of muscle tissue and of skin to the blood samples is to be expected and since the decrease in temperature must be different in various areas of the forearm, the temperature had to be measured under the conditions prevailing in our experiments

To that purpose we proceeded in the following manner A large bore needle fitted with a three way stopcock was used After introducing the needle in one of the cubital veins with its point in the direction of the forearm, the catheter was introduced through the stopcock opening opposite the needle until the tip containing the NTC resistance projected 0 5 cm into the lumen of the vein The catheter was then fixed with a Luer Lok adapter so that no leakage could occur Through the other outlet of the stopcock blood could be sampled. When no samples were drawn the outlet was occluded by a syringe containing a 1 / heparin saline solution The potential of the NTC chain was continuously recorded with the experimental arm extended and relaxed in the usual manner The further procedure was exactly as described for the *in vivo* ultrafiltration experiments. After taking 1 or 2 precompression samples the upper arm was compressed for 30 minutes and 4 to 5 samples taken during this period After releasing the pressure one further sample was taken.

This exp riment was repeated twice in the same arm in the first experiment the needle was introduced into the ulnar cubital vein with the tip of the catheter lying over the brachial artery in the second experiment it was introduced in the median cubital vein with the catheter tip lying just lateral from the tendon of the biceps In a third experiment no blood was sampled but the NTC catheter was introduced in the median cubital vein and pushed 5 cm in the direction of the hand. The results of the three experiments are shown in fig. 31 For the first 2 experiments two temperature curves are shown one indicates the temperature recorded during the time that no samples were taken the other the temperature measured during sampling.

The first two experiments demonstrate that the temperature recorded during sampling differs from that recorded when no blood flows through the vein in which the NTC resistance is situated

Moreover the direction of the temperature change is clearly different at the two sites. Near the brachial artery the precompression temperature is relatively high and practically no change is observed during compression, until during the sampling blood is drawn from peripheral areas Even then the decrease in temperature is not large (—0.8 C). Lateral from the biceps tendon precompression temperature is relatively low and during sampling somewhat lower still But during compression the temperature first increases until after 10 minutes it starts to decrease. This pattern is most marked during sampling. Although during compression the temperature of the blood samples decreases as ex

$Cl + HCO_3$) by extrapolating the experimentally determined regression line to zero protein concentration will represent RA *without* NEFA. By subtracting this value from the value found for (cat min $Cl + HCO_3$) at 100 g/Kg H_2O protein we neglect the contribution of NEFA to the increase in (cat min $Cl + HCO_3$) and overestimate NCE by 0.8 to 0.9 mEq/100 g protein.

However this estimate is based on the assumption that NEFA concentration is determined by protein concentration solely. This is not so. It has become increasingly clear that NEFA is an important source of energy for oxydative processes in muscle tissue (GOODMAN & GORDON 1958) apparently also in the relaxed forearm (ANDRES *et al* 1956, RABINOWITCH & ZIERLER 1962). A decrease in blood flow might therefore result in an increased A-V difference for NEFA. During *in vivo* ultrafiltration this would counteract the effect of the increase in protein concentration on the NEFA concentration in venous blood. The situation is further complicated by the fact that, whereas muscle tissue takes up NEFA, adipose tissue at the same time releases NEFA. The combined effect of these tissue activities is such that the A-V difference for NEFA can become negative especially when blood is sampled from veins draining skin areas (BALTZAN *et al* 1962). Since it is at present not possible to predict the net effect of the three factors mentioned, we will assume that during the *in vivo* ultrafiltration experiments the concentration of NEFA in the venous blood did not change. The available evidence suggests that by this we are more likely to have overestimated NCE (by maximally 1.0 mEq/100 g protein) respectively underestimated RA (by 0.6 mEq/Kg H_2O) than the reverse.

Furthermore, in patients with anaemia, venous lactate concentration may have increased during compression. Neglect of this change will also have caused us to overestimate NCE and to underestimate RA.

(5) *The influence of the Donnan equilibrium can be neglected.* This is true for the determination of NCE, and nearly true for the determination of protein-bound Ca and Mg. In the case of Na however a rather large error is made which presumably results in underestimating protein-bound Na by about 3–4 mEq per 100 g of protein.

(6) *The pH remains constant.* This assumption is not fulfilled and although the change in pH is small, it is not negligible in its effect on our calculations, as will be demonstrated below.

For every plasma a number of straight lines can be constructed, radiating from one point and representing the relation between protein concentration and NCE for different values of pH. This is shown in fig. 32 for a hypothetical plasma containing 6 mEq/Kg H_2O rest anions. The graph was constructed on the assumption that NCE at pH 7.40 is 24 mEq per 100 g protein, and changes 1.0 mEq/100 g protein/0.1 pH unit (VAN SLYKE *et al* 1928).

Although the absolute value for NCE found by Van Slyke *et al* has never been corroborated, the change per unit pH seems reasonably well established (chapter II).

The regression lines calculated from our experiments, however, are different in that at the lowest protein concentration (the pre- and postcompression samples) the pH is higher than at the highest protein concentration (the endcompression samples). The experimental points do not therefore lie on a constant pH line, but – because of the roughly

(b) The direction of the change in temperature occurring during compression depends on the site of sampling and apparently it can be as large as 2 C. But on the average in our experiments the difference between the mean temperatures before and after and the temperature at the end of venous compression must have been less than 1 C, and this probably a decrease This would tend to exaggerate the average decrease in pH during compression by 0 01 It is very unlikely that the error is larger but quite possible that it is smaller and with opposite sign Although therefore in the individual case one must reckon with an error of about 0 03 in measuring the pH change *during* compression, the average error for all experiments must have been negligible

FINAL EVALUATION OF THE ASSUMPTIONS MADE WITH REGARD TO THE DATA OBTAINED BY MEANS OF IN VIVO ULTRAFILTRATION

After discussing the changes observed during compression we are now in a position to consider their possible effect on the calculation of protein-bound cation and of the NCE of the plasma proteins

As stated before the treatment of our experimental data rests on the following suppositions

(1) *The correlation between the concentration of cation e q (cat min Cl + HCO_3) and of plasma protein is linear for protein concentrations ranging from 0 to 130 g/Kg H_2O* From earlier experiments including equilibrium dialysis we already concluded (VAN LEEUWEN *et al* 1961) that this is true for Ca, Mg and Na, but nor for K From the fact that during the course of an *in vivo* ultrafiltration experiment the change in the concentration of (Cl + HCO_3) is remarkably small, it is already to be expected that (cat min Cl + HCO_3) should also show an essentially linear relation with protein concentration Experimental evidence to be presented in chapter VI and VII indicates that this assumption is sufficiently correct to use the method of extrapolation for calculating NCE in the manner described

(2) *The protein composition does not change* This appears to be well established

(3) *Protein-protein interaction can be neglected* The fact that the various plasma proteins differ greatly in their IEP leaves open the possibility that the negative charge on some proteins is balanced by positive charge on others This point will be discussed in some detail in chapter VII where we shall present experimental evidence for the conclusion that in our experiments this effect can be neglected

(4) *The concentration of RA remains constant throughout the experiment* The concentration of lactate and inorganic phosphate was indeed remarkably constant in normal subjects and there is no *a priori* reason to expect a change in the concentrations of SO_4 citrate and the trace anions For the non-esterified fatty acids (NEFA), however the situation must be different since these substances are completely bound by plasma protein The contribution of NEFA to RA should therefore change from nil at a protein concentration zero to about 0 9 mEq/Kg H_2O at a protein concentration of 100 g/Kg H_2O If no other factors influence NEFA concentration the value found for (cat min

Therefore
RA corrected

$$A - x \text{ (mEq/Kg } H_2O)$$

NCE corrected

$$(B - A) + (x - z) \text{ (mEq/100 g protein)}$$

The corrected NCE value must be related to the endcompression pH

The smaller the percentage increase in protein concentration has been, the greater the necessary correction will be.

Assuming the NCE of 100 g protein to change 1 0 mEq per 0 1 pH change (see chapter II) we have calculated for all 34 experiments in normal subjects the value of x and of (x — z). It was found that correction of the error due to the change in pH during compression resulted in, on the average 0.9 mEq/Kg H_2O decrease in the value found for RA, and 0.6 mEq increase in NCE per 100 g protein

For Ca a reasonable estimate of these corrections can be made from the data of TORIBARA *et al* (1957) and of HOPKINS *et al* (1952). Assuming the normal serum or plasma in their experiments to have contained 70 g protein per litre, the work of the authors first mentioned indicates that in the physiological pH range Ca bound per 100 g protein changes 0 12 mEq per 0 1 unit pH whereas from the data of Hopkins *et al* this value is calculated at 0.10 mEq Accepting therefore for Ca bound per 100 g protein a change of 0 11 mEq per 0 1 unit pH the average value of the extrapolation error due to the pH range is for our group of normal subjects calculated at + 0 09 mEq/Kg H_2O for 'free Ca, and at —0.06 mEq for Ca bound per 100 g of protein For Mg the absolute values for the correction must be even smaller and well within the limits of the accuracy of chemical determination.

For Na only a guess can be made, but the absolute value of the error should be somewhat lower than that calculated for NCE.



Summary of chapter IV

When executed nd interpreted in the manner described in this chapter the method of *in i o* ultrafiltration can be used to determine the NCE of the plasma proteins and protein-bound Ca and Mg with reasonable accuracy A correction can be made for the error caused by the (small) decrease in pH occurring during compression. The assumption that the concentration of RA does not change during the experiment may lead to overestimating the value of NCE to a small extent (maximally 1 mEq/100 g protein), at least in normal subjects. In patients with anaemia the overestimate of NCE may be larger as a result of an increase in blood lactate during compression. The neglect of the Donnan equilibrium does not affect the determination of NCE and it will cause protein-bound

linear relation between decrease in pH and increase in protein concentration – are spread in a linear fashion over a number of iso-pH lines. By drawing a straight line through the experimental points and extrapolating to protein concentration zero, an error is made which leads to overestimating RA and to underestimating NCE. This is demonstrated by the gross line in fig. 32 which indicates the calculated regression line for a hypothetical experiment with a precompression protein concentration of 70 g/Kg H_2O at pH 7.35 and an endcompression protein concentration of 110 g/Kg H_2O at pH 7.28

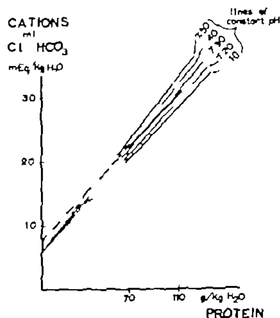


Fig. 32 Systematic error in the determination of NCE and RA resulting from the small decrease in pH during compression See text

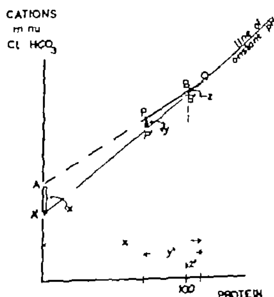


Fig. 33 Scheme explaining correction of the systematic error in NCE and RA caused by the pH change during compression. See text

Correction for error due to change in pH It is possible to correct for this error in the manner demonstrated in fig 33 (which is drawn out of proportion for the sake of clearness) Here P and Q represent the extreme points in one experiment. They differ in pH The straight line drawn through both P and Q is the regression line from which with our mode of calculation RA (indicated as A) and NCE per 100 g pro ein (indicated as B–A) are obtained The other line drawn through Q represents the regression line which would have been obtained if for all experimental points the pH had been the same as at Q The lowest point on the experimental curve would then have been P^1 instead of P A^1 thus represents the true concentration of RA ($B^1 - A^1$) the true NCE *for the pH measured at Q i e the endcompression pH* It will be evident that y (= $P - P^1$) can be calculated from the difference between the pH at P and at Q the protein concentration at P and the change in NCE per 100 g protein and per unit pH Furthermore

$$A - A^1 = x = \frac{x^1}{y^1} \; y \text{ (in mEq/Kg } H_2O)$$

and

$$B - B^1 = z = \frac{z^1}{y^1} \; y \text{ (in mEq/Kg } H_2O).$$

Therefore
RA corrected

$$A - x \text{ (mEq/Kg } H_2O)$$

NCE corrected

$$(B - A) + (x - z) \text{ (mEq/100 g protein)}$$

The corrected NCE value must be related to the endcompression pH

The smaller the percentage increase in protein concentration has been the greater the necessary correction will be.

Assuming the NCE of 100 g protein to change 1.0 mEq per 0.1 pH change (see chapter II) we have calculated for all 34 experiments in normal subjects the value of x and of $(x - z)$. It was found that correction of the error due to the change in pH during compression resulted in, on the average, 0.9 mEq/Kg H_2O decrease in the value found for RA, and 0.6 mEq increase in NCE per 100 g protein.

For Ca a reasonable estimate of these corrections can be made from the data of TORIBARA *et al* (1957) and of HOPKINS *et al* (1952). Assuming the normal serum or plasma in their experiments to have contained 70 g protein per litre, the work of the authors first mentioned indicates that in the physiological pH range Ca bound per 100 g protein changes 0.12 mEq per 0.1 unit pH whereas from the data of Hopkins *et al* this value is calculated at 0.10 mEq. Accepting therefore for Ca bound per 100 g protein a change of 0.11 mEq per 0.1 unit pH the average value of the extrapolation error due to the pH range is for our group of normal subjects calculated at + 0.09 mEq/Kg H_2O for 'free' Ca, and at —0.06 mEq for Ca bound per 100 g of protein. For Mg the absolute values for the correction must be even smaller and well within the limits of the accuracy of chemical determination.

For Na only a guess can be made, but the absolute value of the error should be somewhat lower than that calculated for NCE.



Summary of chapter IV

When executed and interpreted in the manner described in this chapter the method of *in vivo* ultrafiltration can be used to determine the NCE of the plasma proteins and protein-bound Ca and Mg with reasonable accuracy. A correction can be made for the error caused by the (small) decrease in pH occurring during compression. The assumption that the concentration of RA does not change during the experiment may lead to overestimating the value of NCE to a small extent (*maximally 1* mEq/100 g protein), at least in normal subjects. In patients with anaemia the overestimate of NCE may be larger as a result of an increase in blood lactate during compression. The neglect of the Donnan equilibrium does not affect the determination of NCE and it will cause protein-bound

Ca and Mg to be underestimated by about 0 2 and 0 05 mEq/100 g protein only For protein bound Na however the resulting underestimate must be of the order of 3 0 to 4 0 mEq/100 g protein

The method can not be used for the determination of protein-bound K.

Finally our experiments confirm the observation of previous authors that the study of venous forearm blood before and during upper arm compression provides useful data concerning the metabolism of the forearm tissues.

Equilibrium dialysis

When a protein-containing and a protein-free solution are in equilibrium with each other through a semipermeable membrane, the same molal concentration will be found in both solutions for all filtrable substances not affected by the presence of protein. If on the other hand a substance is bound by protein, its concentration will be higher in the protein-containing solution (MICHAELIS & RONA 1908). Although in the case of electrolytes the Donnan equilibrium is a complicating factor, data obtained by equilibrium dialysis can in principle be used to calculate the amount of protein-bound ion, as well as the fraction that is bound in a complex type manner (chapter I).

In our experiments plasma, serum or a solution containing a purified protein fraction was dialysed at 37.5°C against a synthetic 'interstitial fluid' using cellulose tubing for a semipermeable membrane. The dialysis solution contained Na, K, Ca, Mg, Cl, HCO_3, CO and inorganic phosphate in physiological concentrations. By using a closed system technique the ratio HCO_3/CO_2 could be varied and dialysis performed at different pH values. First technique, apparatus and treatment of the experimental data will be described. Thereafter errors in the technique and the observations will be discussed.

TECHNIQUE AND APPARATUS

GENERAL OUTLINE

Covered polyethylene containers were filled with dialysis solution having a temperature of 37°–38°C. The containers were mounted on a shaking device inside an incubator (temperature of the latter 37°–38°C) and the pH of the dialysis solution was adjusted by adding appropriate amounts of concentrated HCl. Then the actual dialysis units consisting of a cone-shaped rubber stopper with a bag of cellulose tubing attached to its lower end, were fitted into holes in the covers of the containers so that the bags hung down into the dialysis solution. The bags were subsequently filled with ± 12 ml of the protein-containing fluid to be studied via a laboratory cannula through a narrow bore channel in the rubber stoppers. One dialysis unit was always filled with distilled water. Immediately after withdrawing the cannula the stoppers were sealed with hardwood plugs and the shaking platform was set in motion. After a period of about 17 hours dialysis was terminated and 1 to 3 samples were taken from the dialysis solution and 1 from the contents of each dialysis unit, using again cannulae and Luer Lok syringes. The transfer to the syringes was done anaerobically with the containers still in the

incubator During the whole procedure precautions were taken to keep bacterial contamination of the contents of the containers minimal

All samples were analysed for Na, K, Ca + Mg, Cl total CO_2, pH and protein in the manner described in chapter III Inorganic phosphate and Ca were not routinely determined One sample of the dialysis solution and at least one sample of the protein-containing fluid were analysed for water content.

Dialysis generally lasted from 16 p m to 9 a.m In some experiments we used one large container with 10 dialysis units, but in most experiments 3 smaller containers were used simultaneously each accommodating 4 dialysis units and each with a different pH value of its dialysis solution In our experiments the pH ranged from 6 3 to 8 0

DETAILS CONCERNING APPARATUS AND ITS PREPARATION

The polyethylene containers

They are of the ordinary commercial type used in refrigerators The covers are flexible and fit clos ly by means of a groove which slips over a rim on the outside of the container By sealing the connection with several layers of adhesive tape leakage can be prevented completely Two sizes of container are used which have the same height (7 cm) but a capacity of 700 ml and of 1300 ml respectively In the covers round holes (1.5 cm diameter) have been punched out 5 in the case of th small containers and 11 in the case of the large one

The containers w re prepared for use in the following manner After carefully fitting the covers on to the containers the latter were thoroughly rinsed with hot tap water filled to overflowing with 1 / chlorhexidin solution and the holes stoppered with clean rubber stopp-rs They were then left to stand for at least 24 hours. Immediately before use the surface of the cover was cleaned with gauze soaked in 1 / chlorhexidin solution the stoppers were removed and the containers emptied by decanting After at least 2 fillings with boiled distilled water and a final rinsing, the containers were filled with the dialysis solution which had b--n warmed to a temp-rature of about 38 C The containers were then stopp-red again placed in th incubator and further prepared for dialysis as described in the general outline After completing an exp-riment the cor tainers wer thoroughly clean-d and prepared again in the manner described

The fact that 1 to 3 ml fluid still remain in the covered containers after the most careful decanting is of no consequence. After 2 fresh fillings with water the chlorhexidin concentration n the 3 ml remaining after the last decanting is 1 p p.m. and the final concentration of chlorhexidin in the 500 ml dialysis solution must have been about 0.01 p p.m *i.e* less than 2 10⁻ mM/L.

Dialysis unit

A schematic representation of the small type container with a dialysis unit *in situ* is given in fig 34 The unit consists of

1 cone shaped rubber stopper with a groove just above its lower end and a narrow channel bored through its length

1 piece of seamless cellulose tubing having a length of 8 cm

1 diabolo-shaped glass bead

2 small size rubber bands

1 small cone-shaped piece of polished wood

The dialysis units were assembled immediately before use in the following manner After boiling stoppers, glass beads and rubber bands several times in distilled water each time refreshing the water sterile pieces of dialysis tubing, prepared in the manner to be described, were carefully folded with one end around the lower part of a stopper with the other end around a glass bead and secured by small rubber bands. The assembled units were checked for leakage by filling with (sterile) water under a pressure of about 200 mm Hg, and after draining and collapsing the bags by suction they were fitted one

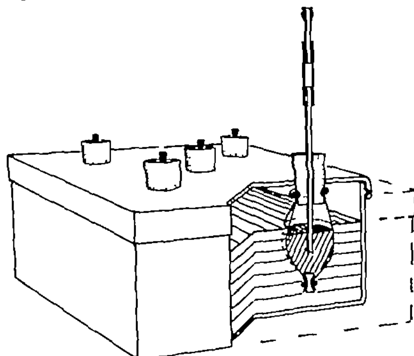


Fig. 14 Polyethylene container with cross-section of dialysis unit and cannula (situation during sampling)

by one into the containers inside the incubator After firmly wedging the rubber stoppers into the holes, the dialysis units were finally filled with the solutions to be dialysed through the stopper channel, which was then plugged with a small cone-shaped piece of polished wood, previously sterilised by boiling. Throughout this procedure the various items were handled with sterile pincers, but the actual assemblage was done manually after the hands had been brushed thoroughly with soap and twice washed with hexachlorophene.

Seamless cellulose tubing (Visking)

Throughout the experiments we used size 32/36 (wall thickness 0.001 inch, average pore radius 24 Å). According to the manufacturer the *cellulose* contains glycerine, water and a small amount of sulphur (approximately 0.1 %) it should be able to withstand steam sterilisation.

The tubing was kept in a polyethylene bag and the day before the start of an experiment pieces of 8 cm length were cut and soaked in distilled water. After some minutes the adhering surfaces could be separated by gently rubbing the tubing between two fingers. The cellulose tubing was then boiled for 20 minutes, rinsed with fresh distilled water and boiled for another 20 minutes. During the night the pieces were left to stand in distilled water and boiled for 15 minutes just before assembling the dialysis units. They were never used twice.

The tubing withstood this treatment without becoming unduly vulnerable and without losing its impermeability to serum proteins, since after 20 hrs dialysis against serum at ± 38 C the dialysis solution consistently gave a negative reaction for albumin (as tested by boiling after addition of 1/10 volume of Sörensen's acetic acid acetate buffer; about 0.03 g/L albumin can be detected in this manner).

HUGHES & KLOTZ (1956), using Visking Nojax sausage casing, reported that after heating on a steambath for one hour sufficient sulphur was released to colour the water slightly yellow and give it a distinct odour of sulphur. With the more refined type of tubing used in our experiments no such effect was ever seen.

Cannula

We used 14 gauge 4" laboratory cannulae with a Luer Lok adapter. To prevent air from entering the container when the syringes and cannulae are disconnected, the latter have been severed 3 cm below the Luer Lok adapter and the 2 parts connected by a piece of closely fitting rubber tubing which can be compressed by a spring clip (See fig 34 where the cannula is shown in the position during sampling). Furthermore, to guarantee an equal distribution of the suction force, small holes were drilled sideways near the end of the cannula. After use the cannulae were taken apart, thoroughly cleaned after soaking in hot water, reassembled, sterilised by boiling in distilled water and dried by suction.

Incubator and shaking device

The inner dimensions of the incubator are 50 × 50 × 50 cm. Inside the door a second door of plexiglass has been fitted which can be worked independently. The plexiglass pane is pierced with two holes, each large enough to admit a forearm.

The shaking device consists of a platform which is made to move in a horizontal plane (140 times a minute over a distance of 6 cm) by means of an excentric transmission. The latter is connected with an electromotor outside the incubator via an axle projecting through its back panel. As a result of this construction the temperature of the solution inside a container placed on the platform can be kept constant to 0.5°C. With every experiment the temperature is checked by means of an (alcohol sterilised) mercury thermometer fitted in one of the containers directly after filling the latter with dialysis solution. In the experiments to be recorded the temperature ranged from 36.5 –38.0 C at the beginning of the dialysis to 37.0 –38.0 C at the end. During sampling the temperature inside the containers remained constant to 0.2 C.

Dialysis solution

During the experiments to be recorded the solutions were at first prepared to contain approximately the following concentrations

Na	144.0	Cl	118.0
K	3.5	HCO_3	32.0
Mg	1.5	inorganic phosphate	2.0
Ca	3.0	(at pH 7.4)	
	152.0 mEq/L		152.0 mEq/L

Later the K concentration was increased to 5 mEq by adding more KCl. The solution was generally prepared in amounts of 5 litres and to prevent the formation of $CaCO_3$ the following procedure was adopted. NaCl, KCl, Na HPO and $NaHCO_3$ were dissolved first the latter in a quantity of 35 mEq/L. Then 3 ml 1 N HCl was added per litre, and the fluid gently mixed in a large stoppered flask. As a result of the formation of H_2CO_3 the pH decreased only to about 7.40, and $CaCl_2$ and $MgCl_2$ could then be added without precipitation of $CaCO_3$. A stock of 5 litre solution was sufficient for about 3 dialysis experiments and could be kept in the rubber-stoppered flask at room temperature for a fortnight without precipitation of $CaCO_3$ and without bacterial growth. Just before starting an experiment the quantity needed was decanted into an Erlemeyer flask and – without stirring – heated in a waterbath till it had reached a temperature of 40°C. It was then decanted into the containers (500 $\pm$ 5 ml in the small ones, 1100 $\pm$ 10 ml in the large one) and the containers with the solution immediately placed in the incubator. The pH of the solution was then about 7.80.

Frequent temperature measurements indicated that with this technique the temperature of the solution was between 36 and 38 at the moment of entering the incubator

Then, according to which pH range was to be studied, 0.1 to 0.9 ml of 10 N HCl was pipetted into the containers and the latter set shaking for about 3 minutes. The CO_2 thus liberated escapes and produces a considerable overpressure. By momentarily opening one of the holes in the cover the pressure inside the containers was allowed to become atmospheric again. Subsequently while placing the dialysis units in position, care was taken that only one hole at a time was open. After some experimenting the intended pH decrease of the solution could be realised to within 0.2 pH unit.

This way of proceeding was found more convenient than the use of solutions with different concentrations of bicarbonate equilibrated against gas containing 5% CO_2 + 95% O_2

Since at least one dialysis unit was always filled with distilled water the concentrations in the samples of dialysis solution taken after the dialysis were somewhat lower than those of the stock solution

Distilled water

In each container one dialysis unit was always filled with distilled water This unit served as a control on whether equilibrium had been reached since in that case the concentrations of the filtrable ions will after dialysis be the same for the solution inside the control unit and for the dialysis solution

Protein-containing solutions

Apart from the control unit filled with distilled water the dialysis units were filled with protein-containing solutions In most experiments serum was used, in some plasma. A number of experiments were also done with solutions of purified albumin and γ-globulin These will be specified when we present our results in chapter VII

Serum and plasma were obtained in the following manner within 3 hours before starting the dialysis about 110 ml blood was collected in a sterile small model transfusion flask using the ordinary needle set for donors. When plasma was needed 0 1 ml 5 % heparin solution was added beforehand, which made the final concentration of heparin in the plasma between 0 005 to 0 01 %. The flask was centrifuged for 60 minutes (2500 rpm at 30°C) and the supernatant pipetted into an Erlemeyer flask, care being taken that sterile glassware was used throughout. The 50–60 ml serum or plasma obtained were stored at 5 C until they could be transferred to the dialysis unit by means of sterile syringes and cannulae. The maximum capacity of the dialysis bags was about 15 ml, but in the experiments they were filled with $\pm$ 12 ml fluid In some experiments the protein-containing fluid was diluted to a varying extent with dialysis solution in order to study the effect of changes in protein concentration

In previous experiments and in the earlier ones of those presented in this study plasma was used. As fibrin frequently formed during the dialysis, only serum was used in the later experiments. With it no late formation of fibrin occurred, at least not visibly The possibility of differences resulting from the use of either plasma or serum will be discussed when we consider the errors of the method.

The meaning of the terms dialysis solutions, distilled water' serum and plasma

The solutions in the various compartmens of a container will change in composition during dialysis. Henceforward the terms dialysis solution distilled water serum or plasma will refer to the solutions obtained *after dialysis* unless otherwise stated The term distilled water is placed between quotation marks since after dialysis the solution inside the control units obviously no longer is distilled water

SAMPLING AND TREATMENT OF ANALYTICAL DATA

It was already mentioned that after dialysis the containers were left to stand in the incubator and that sampling was done with the plexiglass door closed When the large container was used 2 (sometimes 3) samples of dialysis solution were taken *i.e.* the first (the fifth) and the last of all samples taken from the container In the case of the small containers only 1 sample of dialysis solution was obtained and this was the first of the samples taken The control unit was sampled sixth in sequence in the case of the large container and last in the case of the small containers.

After chemical analysis of the samples and calculating HCO_3 from total CO_2 and pH

all concentrations were expressed per Kg H_2O and for each sample (cat min Cl + HCO_3) was calculated

For each experiment the correlation between protein concentration and water content was derived from the samples for which water content was determined, i.e. always 1 sample of dialysis solution and at least 1 sample of protein-containing solution (see chapter VI).

Then for Na, K, Ca, Ca + Mg, Cl, HCO_3 and (cat min Cl + HCO_3) the concentration outside the dialysis unit (for which the average concentration in the samples of dialysis solution was taken) was subtracted from the concentration inside each dialysis unit. The values so obtained are indicated by the symbol Δ (see chapter I). They were correlated with the protein concentration inside the unit and expressed in mEq per 100 g protein. The values thus found for Δ (cat min Cl + HCO_3) are considered to represent NCE of the plasma proteins the values found for Δ Na, Δ K, Δ Ca + Mg will be considered as such, since in order to obtain from them the quantity of protein-bound cation they must be corrected for the Donnan equilibrium (chapter I)

Concentration ratios ($[Cat]_o/[Cat]_i$ and $[An]_i/[An]_o$) were calculated for each unit, $[Cat]_o$ and $[An]_o$ representing the average concentration in the samples of dialysis solution. From the ratios actually found and the protein concentration inside the unit, the concentration ratios to be expected at a protein concentration of 70 g/Kg H_2O were calculated for each unit (for details see chapter VII). No concentration ratio was calculated for H-ions, for reasons to be explained presently

SOURCES OF ERRORS IN TECHNIQUE AND OBSERVATIONS

In the following paragraphs we shall discuss the accidental errors and the various sources for systematic errors.

ACCIDENTAL ERROR CALCULATED FROM DIFFERENCES IN THE RESULTS OBTAINED FOR COMPARABLE SAMPLES

This we could calculate for two different solutions i.e the dialysis solution outside and the protein-containing fluid inside the dialysis units.

Dialysis solution

Twenty-five experiments are available in which more than one sample of dialysis solution was taken By subtracting the concentration found in the second sample of dialysis solution from that found in the first sample, the differences in concentration are provided with a sign and the mean value SD and range of the difference can be calculated. As in later experiments only one sample of dialysis solution was taken, the 25 experiments used in calculating table 28 covered the period between May 1960 and May 1961

It can be seen that there is no significant difference between the concentrations obtained for consecutive samples of a particular dialysis solution. Taking into consideration the relatively small number of pairs available, the SD is not much different from that calculated for the duplicate determinations of a plasma sample (chapter III). Special attention should be given to the fact that there is no significant difference in pH between the two samples of dialysis solution.

Distilled water

In each container one dialysis unit was always filled with distilled water This unit served as a control on whether equilibrium had been reached since in that case the concentrations of the filtrable ions will after dialysis, be the same for the solution inside the control unit and for the dialysis solution

Protein-containing solutions

Apart from the control unit filled with distilled water the dialysis units were filled with protein-containing solutions In most experiments serum was used, in some plasma. A number of experiments were also done with solutions of purified albumin and γ-globulin These will be specified when we present our results in chapter VII

Serum and plasma were obtained in the following manner within 3 hours before starting the dialysis about 110 ml blood was collected in a sterile small model transfusion flask using the ordinary needle set for donors When plasma was needed 0.1 ml 5 % heparin solution was added beforehand, which made the final concentration of heparin in the plasma between 0 005 to 0 01 %. The flask was centrifuged for 60 minutes (2500 rpm at 30°C) and the supernatant pipetted into an Erlemeyer flask, care being taken that sterile glassware was used throughout. The 50–60 ml serum or plasma obtained were stored at 5 C until they could be transferred to the dialysis unit by means of sterile syringes and cannulae The maximum capacity of the dialysis bags was about 15 ml, but in the experiments they were filled with ± 12 ml fluid In some experiments the protein-containing fluid was diluted to a varying extent with dialysis solution in order to study the effect of changes in protein concentration.

In previous experiments and in the earlier ones of those presented in this study plasma was used. As fibrin frequently formed during the dialysis, only serum was used in the later experiments With it no late formation of fibrin occurred, at least not visibly The possibility of differences resulting from the use of either plasma or serum will be discussed when we consider the errors of the method.

The meaning of the terms dialysis solutions, distilled water serum and plasma

The solutions in the various compartmens of a container will change in composition during dialysis Henceforward the terms dialysis solution distilled water serum or plasma will refer to the solutions obtained *after dialysis* unless otherwise stated The term distilled water is placed between quotation marks since after dialysis the solution inside the control units obviously no longer is distilled water

SAMPLING AND TREATMENT OF ANALYTICAL DATA

It was already mentioned that after dialysis the containers were left to stand in the incubator and that sampling was done with the plexiglass door closed When the large container was used 2 (sometimes 3) samples of dialysis solution were taken *i e* the first, (the fifth) and the last of all samples taken from the container In the case of the small containers only 1 sample of dialysis solution was obtained and this was the first of the samples taken The control unit was sampled sixth in sequence in the case of the large container and last in the case of the small containers

After chemical analysis of the samples and calculating HCO_3 from total CO_2 and pH

all concentrations were expressed per Kg H_2O and for each sample (cat min Cl + HCO_3) was calculated.

For each experiment the correlation between protein concentration and water content was derived from the samples for which water content was determined, i.e. always 1 sample of dialysis solution and at least 1 sample of protein-containing solution (see chapter VI).

Then for Na, K, Ca, Ca + Mg, Cl, HCO_3 and (cat min Cl + HCO_3) the concentration outside the dialysis unit (for which the average concentration in the samples of dialysis solution was taken) was subtracted from the concentration inside each dialysis unit. The values so obtained are indicated by the symbol Δ (see chapter I). They were correlated with the protein concentration inside the unit and expressed in mEq per 100 g protein. The values thus found for Δ (cat min Cl + HCO_3) are considered to represent NCE of the plasma proteins; the values found for Δ Na, Δ K, Δ Ca + Mg will be considered as such, since in order to obtain from them the quantity of protein-bound cation they must be corrected for the Donnan equilibrium (chapter I).

Concentration ratios ($[Cat]_o/[Cat]_i$ and $[An]_i/[An]_o$) were calculated for each unit, $[Cat]_o$ and $[An]_o$ representing the average concentration in the samples of dialysis solution. From the ratios actually found and the protein concentration inside the unit, the concentration ratios to be expected at a protein concentration of 70 g/Kg H_2O were calculated for each unit (for details see chapter VII). No concentration ratio was calculated for H-ions, for reasons to be explained presently.

SOURCES OF ERRORS IN TECHNIQUE AND OBSERVATIONS

In the following paragraphs we shall discuss the accidental errors and the various sources for systematic errors.

ACCIDENTAL ERROR CALCULATED FROM DIFFERENCES IN THE RESULTS OBTAINED FOR COMPARABLE SAMPLES

This we could calculate for two different solutions, i.e. the dialysis solution outside and the protein-containing fluid inside the dialysis units.

Dialysis solution

Twenty-five experiments are available in which more than one sample of dialysis solution was taken. By subtracting the concentration found in the second sample of dialysis solution from that found in the first sample, the differences in concentration are provided with sign and the mean value, SD and range of the difference can be calculated. As in later experiments only one sample of dialysis solution was taken, the 25 experiments used in calculating table 28 covered the period between May 1960 and May 1961.

It can be seen that there is no significant difference between the concentrations obtained for consecutive samples of a particular dialysis solution. Taking into consideration the relatively small number of pairs available, the SD is not much different from that calculated for the duplicate determinations of a plasma sample (chapter III). Special attention should be given to the fact that there is no significant difference in pH between the two samples of dialysis solution.

Since the mean of the differences did not differ significantly from zero we can consider the SD of the difference given in table 28 to indicate the SD for a single determination of Na etc. in dialysis solution

Serum or plasma

Since for each dialysis unit the difference in ion concentration between the protein-containing solution inside and *the dialysis solution outside is expressed per 100 g* protein the values can be compared regardless of the actual protein concentration inside each dialysis unit *By filling two units from the same serum sample, the effect of accidental errors on the value obtained for Δ Na Δ K etc. can be estimated. But there is a difference according to whether units from one container are compared or units from different containers In the first instance only errors in analysis of the inside solution are detected* since the outside solution – the dialysis solution – is identical for the units in one container If on the other hand results from different containers are compared the final error in Δ Na Δ K etc. includes also the error inherent in the analysis of dialysis solution

(a) *Comparison of results from one container*

In practically all experiments at least two dialysis units were filled with the same (undiluted) serum or plasma. A fairly large number of paired results are thus available for studying *the effect of accidental errors on the values calculated for Δ Na etc.* Since the latter are derived from the differences in concentration inside and outside and multiplied to express them per 100 g of protein the spread must be larger than in the case of the concentrations proper We have calculated this spread in the following manner For two paired dialysis units filled with the same serum or plasma the differences in the final Δ values are calculated by subtracting the values found *for the unit second in* sequence of sampling from those found for the unit sampled first. To detect which of the determinations contributes most to the spread in Δ (cat min Cl + HCO_3), we have *calculated in the same manner the difference between Cl respectively* HCO_3 concentration inside and outside the dialysis unit and expressed it per 100 g of protein For the differences so obtained the mean the SD and range were calculated Results from 54 *experiments are available covering the period from* May 1960 to December 1961 Table 29 summarises our findings.

It will be seen that again no significant difference is found between the paired samples. The SD of the difference can therefore be considered to indicate the SD for a single determination of Δ Na etc. as well

> The actual SD of Δ (cat min Cl + HCO_3) is smaller than that calculated from the SD of the *individual factors* ($\sqrt{0.51^2 + 0.05^2 + 0.10^2 + 0.77^2 + 0.44^2} = 1.1$). This is due to the fact that the spread found for Δ cations is not independent from that found for Δ anions since the errors *in protein determination are the same for both.*

A further point of interest is the fact that *no significant difference* was found between the pH value of the serum-containing-unit sampled first and the one sampled next Finally it must be stressed that the spread indicated in table 29 *is not applicable to the results obtained* for dialysis units containing less than 50 g/Kg H_2O of protein This will be discussed in more detail in one of the following sections.

(b) *Comparison of results from different containers*

Since in this case both inside and outside solution are different for the two units compared, both the errors in analysis of serum and in analysis of dialysis solution affect the difference in the values found for Δ Na etc.

Table 29 refers to the values obtained by multiplying the experimental results to express Δ Na etc. per 100 g protein. In reality however the error in analysis is made when determining the difference between the concentration inside and outside at the actual protein concentration of the solution inside. The latter was on the average 63 g/Kg H_2O for the experiments in table 29 and the spread to be expected in serum analysis proper is therefore 0.63 SD presented in table 29. From the SD so obtained and the SD for analysis of dialysis solution (table 28) one can make reasonable estimate of the SD to be expected for a single determination of Δ Na, Δ K etc. at protein concentration of 63 g/Kg H_2O. The values thus found must be multiplied by 100/63 to obtain the SD to be expected for Δ Na etc. expressed per 100 g protein (table 30).

Strictly speaking the SD given in table 30 only holds for samples containing 63 g/Kg H_2O protein. The lower the protein concentration the higher the SD to be expected, because of the multiplication necessary to express the values per 100 g of protein.

But since the values given in table 29 derive from pairs of units with protein concentrations ranging from 52 to 73 g/Kg H_2O and since the units containing the lowest protein concentration will show the largest spread (and therefore largely determine the SD of the group as a whole), we have accepted the values indicated in tables 29 and 30 as being valid for the experiments in which protein concentration varied between 50 and 75 g/Kg H_2O. We thereby slightly overestimate the accidental error to be expected at protein concentrations higher than 63 g/Kg H_2O.

SYSTEMATIC ERRORS

There are various sources for systematic errors in our experiments. As such we will discuss: The influence of the semipermeable membrane *per se* on ion distribution, the possibility that no equilibrium was reached during dialysis, bacterial contamination, the effect of ageing on ion binding properties of the plasma proteins, differences between plasma and serum, the relation between the actual protein concentration and the values found for Δ Na etc. expressed per 100 g protein, and the fact that no anions other than Cl and HCO_3 were determined.

Influence of the semipermeable membrane per se on ion distribution

Since we have correlated the difference in the *concentration* of filtrable ion inside and outside the membrane with the *concentration* of protein inside, adsorption of ion will have had no effect on our results. Furthermore we found the membrane to be non-selective for all small ions studied (see next section).

Evidence for the existence of a state of equilibrium after 17 hours dialysis

Filtrable ions other than H-ions

In preliminary experiments all dialysis units in a container were filled with distilled water and dialysed for 15 hours at 37.5 C against the dialysis solution described before. At the end of that time the solutions inside and *outside* the bags were always equal, within the error of determination, as regards the concentrations of Na, K, Mg, Ca,

of this characteristic of serum, the above mentioned explanation does not answer the question concerning the relatively high pH of serum samples as compared to dialysis solution We must therefore envisage the possibility that a slight difference in measured pH results from a change in the junction potential when serum is measured instead of saline solution. The above mentioned observations explain why we had to abandon our original intention to use the concentration ratio of H ions as indicator of the Donnan ratio

All this has no effect on the determination of Δ (cat min Cl + HCO_3) since in the case of HCO_3 the mean of the differences in concentration does not differ significantly from zero and the distribution of the individual values is symmetrical (fig. 35 below) This

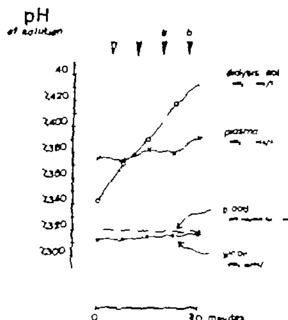


Fig 36 – Effect of contact with air and of partial vacuum on pH of different solutions.
( ) 2 ml air in syringe containing 5 ml fluid. Expulsion of air after gentle mixing
(b) 0.5 ml air in syringe containing 4 ml fluid. Creation of partial vacuum by pulling out plunger to 8 ml mark. Expulsion of air

finding might be interpreted as an argument in favour of the hypothesis that CO_2 occasionally escapes from the solution during sampling since in that case the resulting decrease in total CO_2 content and the concomitant increase in pH cancel each other in the calculation of the HCO_3 concentration

Bacterial contamination

All bacteriological examinations were done by Miss J Fass in our Bacteriological Laboratory which is under the direction of Dr N Lubsen.

The conditions during dialysis are ideal for bacteriae and the more or less heavy growth of *Ps aeruginosa* observed in most experiments was not altogether unexpected But n other bacteriae were ever cultured and we were lucky in that the bacteriae were only found in the dialysis solution the contents of the dialysis units remaining remarkabl free This suggests that the aseptic precautions during assembling and filling of the units were adequate and that the dialysis solution was contaminated from either of three sources the containers, the inside of the incubator and the material from which the dialysis solution was prepared.

The containers seemed highly improbable as a source, since chlorhexidin in a concentration of 1 : 50,000 was found to be bactericidal for this strain of *Ps. aeruginosa*. The inside of the incubator could also be excluded, as cultures from swabs taken from all inner surfaces were consistently negative. Moreover, repeatedly washing the inside of the incubator with lysoform did not prevent the growth of *P. aeruginosa* during dialysis. Contamination of the stock of dialysis solution appeared also to provide no explanation since in earlier experiments the dialysis solution was autoclaved after its preparation and yet *Ps. aeruginosa* was found at the end of dialysis. We stopped autoclaving the dialysis solutions as being an useless increase in labour and subsequent cultures from the 5 litre stock of dialysis solution always remained negative. Towards the end of the experiment, when all other precautions remained without effect, we again autoclaved the stock solution: the first time without result, the two last times no bacterial growth was observed. Since occasionally also no growth occurred while using non-sterilised stock of dialysis solution, we are as yet not certain whether the contamination really occurs during the preparation of the dialysis solution. But after having tried all other measures without avail we are now inclined to think it does. The fact that direct cultures from the stock solution always gave negative results must be explained by the fact that the bacterial density in the dialysis solution remains extremely low until it is dialysed at 38°C and small amounts of nitrogen containing substances (urea) and of glucose diffuse from the serum.

Attempts to prevent bacterial growth by adding oxytetracyclin (30 mg/L) to the dialysis solution were not successful because at 38°C and pH 7.4 oxytetracyclin is broken down within three hours. As *Ps. aeruginosa* was resistent to the other antibiotics available at the time, we also tried NaN_3 (0.5 ‰), but without success. Chlorhexidin in a concentration of 1 : 5000 effectively inhibited the growth, but was found to result in a 10–20 % error in the determination of Ca and Mg.

For practical purposes it should be mentioned that the macroscopic aspect of the solution was found to be a reliable guide in detecting bacterial growth as the latter always caused slight opalescence. If the solution was crystal-clear both the Gram stain of the sediment and the cultures were without exception negative.

Although in most of our experiments we were unable to prevent growth of *Ps.aeruginosa* in the dialysis solution, the following points suggest that this has had no effect on our results.

In practically all experiments the contents of the dialysis units remained free from bacteriae and since *Ps. aeruginosa* is not known to produce filtrable proteolytic enzymes, it is improbable that the protein inside the dialysis units was altered by the bacterial growth outside.

Even in the presence of heavy growth of *Ps. aeruginosa* the potentiometric determination of Cl was not affected. Moreover, as already mentioned, equal concentrations were found for all substances inside and outside the control dialysis unit filled with distilled water, although the solution inside was always free from bacteriae.

The effect of ageing on the ion binding properties of the plasma proteins

Normal plasma contains proteolytic enzymes and it is conceivable that these change the number of ionogenic groups on the proteins during the nearly 24 hours dialysis at about 38°C and at physiological pH. We have therefore dialysed serum both when fresh and after it had been stored 1, 3 and/or 7 days at 5°C (3 sera) or at 38°C (1 serum). The results are summarised in fig. 37 and need some explanation.

In view of the effect of pH on the values obtained for Δ Na etc. the consecutive dialyses of a given serum should have been performed at exactly the same pH value. But since with our technique the pH realised in a given container sometimes differed as much as 0.2 pH units from the value intended, fig. 37 shows the values obtained for Δ Na, Δ Ca + Mg and Δ (cat minus Cl − HCO_3) in relation to the pH values during dialysis.

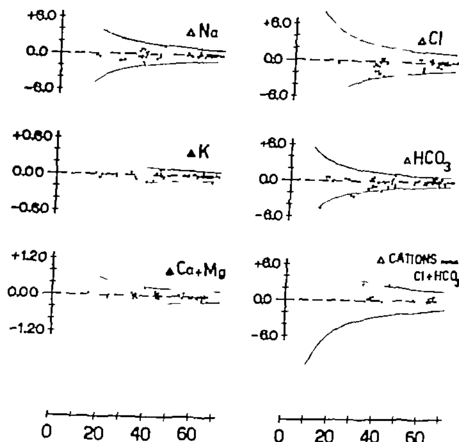


Fig. 38 Influence of actual protein concentration inside dialysis unit on the Δ values expressed in mEq/100 g protein.

Each point represents one dialysis unit the zero lines indicate mean of Δ values obtained for undiluted serum the two curved lines accompanying each zero line enclose the area covered by ± 2 SD for single point.

The relation between the actual protein concentration and the values for Δ Na, Δ K etc. expressed per 100 g protein

When we express Δ Na etc per 100 g protein we tacitly assume that there is a rectilinear relation between protein concentration and Δ Na etc If this assumption is correct the *actual* protein concentration inside the dialysis unit will have no influence on the values calculated for Δ Na etc per 100 g protein We have therefore done 10 experiments in which undiluted serum as well as various dilutions of the same serum were simultaneously dialysed in one container

In each experiment two units contained undiluted serum, the actual concentration of the latter varying from 55 to 71 g/Kg H_2O The serum dilutions were made by adding dialysis solutions to the serum. The pH during dialysis varied between 7 10 and 7.50.

The results obtained are presented in the following manner For each dialysis unit in one container the Δ values (in mEq/100 g protein) were subtracted from the mean of the

Δ values (in mEq/100 g protein) obtained from the two units with undiluted serum in the same container. The differences were plotted against the actual protein concentration in the dialysis unit. The results are for the 10 experiments together shown in fig. 38, where each point represents one dialysis unit.

At first sight the points appear to be equally distributed around the zero line for each of the Δ values. In the lower range of the actual protein concentrations, however, the scatter is considerable. This is explained by the fact that the error due to errors in analysis is magnified by expressing the Δ values per 100 g protein. The inevitable increase in spread is the larger the lower the actual protein concentration. In order to know whether the scatter observed is within the range to be expected from errors in analysis we proceeded as follows.

From table 29 we know the SD to be expected for Δ Na etc. (expressed per 100 g protein) when the actual protein concentration is 63 g/kg H_2O and when samples from one container are compared. If we make the assumption that the absolute errors in electrolyte determination are independent from the protein concentration inside the dialysis unit we can calculate the SD to be expected for an actual protein concentration X by multiplying the SD given in table 29 by the factor 63/X. In this manner the curved lines have been constructed which in fig. 38 accompany each zero line. They indicate the range covered by plus or minus 2 SD to be expected for a single point.

Three points (2 for Δ Ca + Mg, 1 for Δ K) differ more than 3 SD from the zero line and these may therefore be due to gross error. All other points are within or nearly within 2 SD from the zero line. But whereas for Δ Na, Δ K, Δ Ca + Mg and Δ HCO_3 the points are equally distributed around the zero line over the whole protein range, they tend to deviate in the lower range of protein concentration for Δ Cl and consequently also for Δ (cat min Cl + HCO_3).

We have therefore also calculated the mean deviation from the zero line for the points within the protein ranges 10–20, 20–30, 30–40 and 40–50 g/Kg H_2O respectively. For each range the resulting value was compared with 2 SE of the mean to be expected at the average protein concentration for that range. For all other Δ values the mean deviation from the zero line was within 2 SE of the mean for each of the protein ranges, but for Δ Cl and for Δ (cat min Cl + HCO_3) the mean deviation was between 2 SE and 3 SE of the mean in the protein ranges 10–20 and 30–40 g/Kg H_2O (table 34). The number of points in the lowest range however is only five and in view of the manner in which we had to calculate the SD to be expected at the various protein concentrations we must conclude that the number of experiments is too small to decide whether the observed deviation is statistically significant. If there really is a deviation for Δ Cl to lower values and for Δ (cat min Cl + HCO_3) to higher values when the actual protein concentration decreases, the effect should be to underestimate Δ Cl and to overestimate Δ (cat min Cl + HCO_3) by expressing them per 100 g protein. However the trend of the points in fig. 38 and the values in table 34 suggest that for experiments with undiluted serum the error would still be less than 0.3 mEq/100 g protein.

Since henceforward we will only consider results obtained with dialysis units containing more than 50 g/Kg H_2O protein we conclude that in the experiments to be presented later the expression of Δ Na, Δ K, Δ Ca + Mg, Δ HCO_3, Δ Cl and Δ (cat min Cl + HCO_3) in mEq/100 g protein did not introduce a systematic error. This is equal to saying that the relation between protein concentration and Δ Na etc. is rectilinear for protein concentrations between 50 and 100 g/Kg H_2O.

Errors due to the fact that no anions other than Cl and HCO_3 were determined

The one other anion present in the stock dialysis solution phosphate, was determined only occasionally because of the already considerable amount of analyses However since both its concentration in the stock solution (2.7–2.9 mg %) and the pH are known, the phosphate concentration in a sample of dialysis solution can be estimated to within 5 % at pH 7 40 it should be 1 6 ± 0 1 mEq/Kg H_2O Furthermore, during dialysis of serum or plasma the filtrable rest anions other than phosphate will be distributed over the total volume of fluid in the container and since maximally 60 ml of serum was dialysed

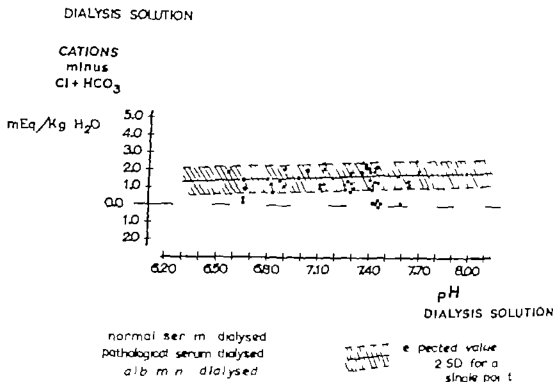


Fig. 39 – V lues observed compared with values expected for (cat min Cl + HCO_3) in dialysis solution. See text.

against 1100 ml dialysis solution the final concentration of these anions is at the most 0 05 times the original serum concentration If we assume the latter to be 4 0 mEq/Kg H_2O (chapter II) the final concentration of filtrable anions other than Cl and HCO_3 can be estimated at 1 8 mEq/Kg H_2O for pH 7 40. We must therefore expect to find this value for (cat min Cl + HCO_3) in both dialysis solution and distilled water when normal serum is dialysed. Its magnitude is slightly different at other pH values because of the effect of the latter on the dissociation of inorganic phosphate

In fig. 39 the value expected for (cat min Cl + HCO_3) in the dialysis solution is compared with the value actually found in the 69 experiments in which plasma, serum or purified protein solutions were dialysed The continuous line indicates the expected value, each point the actual value found for the dialysis solution The SD for a single point should be 0 40 mEq/kg H_2O (see table 28), and the hatched area between the broken lines in fig 39 encloses all values which differ not more than twice the SD from the value expected.

It is evident that only half the number of experimental points fall within the probable range. Some are higher but most are lower than the expected value and some even carry a negative sign! That these 'aberrations are greater than the ordinary spread can explain, is corroborated by the fact that in the case of the three extreme values +4 6 —1 1 and —2.0, the values found in the paired samples of distilled water' were +6.0, —1 1 and —2.2 respectively Together with the results summarised in table 31 this demonstrates that the deviation from the expected value is found both inside and outside the dialysis units, and to an equal extent. The cause(s) of this phenomenon is (are) not clear

In view of the data presented in chapter III it is improbable that the discrepancies observed are the result of differences between the standard solutions used for the determination of the cations and of Cl and HCO_3. Moreover quite often one of the three containers used during one dialysis experiment would yield a value of for instance +2.0 for (cat min Cl + HCO_3), whereas the values found in the other two containers would be zero or even negative. Initially we thought that the differences observed were explained by summation of accidental errors in analysis, but when it became clear that the reproducibility was considerably better than expected, w determined inorganic phosphate in number of experiments to exclude the possibility of errors from that quarter Without exception the concentration of inorganic phosphate was found to be within the range 2 6–2.8 mg %, although in the same samples the value for (cat min Cl + HCO_3) ranged from 0 0 to 3.9 mEq/Kg H_2O Three of the higher values were found in an experiment with serum from patient with multiple myeloma, but the rest anions provided no explanation as their concentration in the original plasma was found to be not more than 8 mEq/Kg H_2O (as ... ultrafiltration method)

If we reject systematic errors in chemical analyses (see chapter III) only the presence of an unknown cationic substance (when (cat min Cl + HCO_3) is lower than expected) or anionic substance (for the values higher than expected) remains as an explanation.

It is possible that the solutions sometimes contained small amount of NH_4-ions at the end of the dialysis, but it is unlikely that this ion can reach the concentration of 2 3 mEq/Kg H_2O necessary to explain the negative values for (cat min Cl + HCO_3), which were occasionally observed. W could find no correlation whatsoever between the presence of *Ps. aeruginosa* in the dialysis solution and the phenomenon under discussion. This bacteria is not know to produce ammonia in any case If the ammonia resulted from desamination of proteins by plasma enzymes, this process should have to produce 2 3 millimoles of NH_4-ions from substrate of about 4 g of protein and 20 mg (= 0 3 millimole) of urea. This would mean that 40 millimoles of NH_3 should be split off from 100 g protein change hich should have had considerable influence on NCE. The results of the experiments with stored serum or plasma argue against such an occurrence.

Possibly filtrable cationic substance was released from the alls of the containers. W hav done those experiments in which the containers were filled with NaCl solution or with dialysis solution only The results of the experiments in which saline was used have already been presented for other reasons in chapter III in the section on Cl determination, but no evidence for the release of an unknown ion was found Table 35 summarizes the results of another experiment in which 2 containers were shaken ith dialysis solution for 18 hours. I this experiment inorganic phosphate was determined lso. In both containers the change in (cat min anions) was less than twice the SE of the mean.

We have as yet no explanation for the observed deviation of (cat min Cl + HCO_3) from the value expected. But the fact that sign and magnitude of the deviation were always the same for dialysis solution and for distilled water suggests that the phenomenon w ll have no nfluence on our calculation of Δ (*cat min Cl + HCO_3*), since the latter is obtained from the difference between the value found inside and the one

found outside a dialysis unit In chapter VII we will nevertheless consider separately the results obtained in the experiments in which (cat min Cl + HCO_3) of the dialysis solution was within the probable range and in those in which (cat min Cl + HCO_3) was clearly outside that range

Apart from the question of their *total concentration* there is the question of the distribution of the anions not determined by us. It will be evident that Δ (cat min Cl + HCO_3) can only be interpreted as representing NCE if the concentration of all other anions is equal inside and outside the dialysis unit. In view of the effect of the Donnan equilibrium on the one hand and the possible existence of protein-bound rest anion on the other hand we will have to be more explicit on this point.

Because of the Donnan equilibrium one would expect the concentration of phosphate (expressed per Kg H_2O) to be lower inside the serum-containing unit However we already discussed in chapter IV the observations of others who found in dialysis or ultrafiltration experiments either an increased concentration at the protein-containing side of the semipermeable membrane (GREENE & POWER 1931 WALSER 1961) or an equal concentration on both sides (HOPKINS *et al* 1952). SEEKLES (1936) who worked with bovine serum could find no clear evidence of protein-bound phosphate Our own results with *in vivo* ultrafiltration agree best with the observations of Hopkins *et al* and indicate that at phosphate concentrations up to 10 mg / and under the conditions of *in vivo* ultrafiltration the effects of binding by protein and of the Donnan equilibrium cancel each other Furthermore we observed in one dialysis experiment with normal dialysis solution no significant difference between the phosphate concentration inside and outside the protein-containing dialysis units (table 36) This table also includes the results of 3 experiments with a dialysis solution containing a fairly high concentration of phosphate buffer instead of the usual bicarbonate/CO_2 mixture. The solution contained normal concentrations of Na, K and Cl but no Ca or Mg. It can be seen that at these high concentrations the phosphate content on the inside was slightly lower than on the outside but the difference was not more than 4 %. Walser found that the percentage of inorganic phosphate presumably bound by protein is highest if the phosphate concentration is low We can therefore conclude that at the concentrations used in our experiments there is no significant difference between the concentration of inorganic phosphate inside the serum-containing units and that outside them



Summary of chapter V

Δ Na, Δ K Δ Ca and Δ Ca + Mg could be determined with reasonable accuracy and expressed in mEq/100 g protein However to obtain from these values the quantity of protein bound cation a correction must be made for the effect of the Donnan equilibrium as the latter causes the concentration of 'free' filtrable cations to be lower on the protein containing side of the membrane than on the other side For this correction the Donnan

ratio must be known. The latter was not determined directly but in chapter VIII an attempt will be made to estimate its value from circumstantial evidence.

The accurate determination of Δ (cat min Cl + HCO_3) is rather difficult in view of the cumulative effect of errors in determination and interpretation. But the available evidence indicates that in the case of the units containing at least 50 g/Kg H_2O protein the systematic error – if present – must have been small (less than 1 mEq/100 g protein) and must have led to overestimating rather than underestimating Δ (cat min Cl + HCO_3) The evidence further suggests that the latter is equal to the NCE of the proteins in the experiments in which serum was dialysed. When plasma was dialysed the heparin present may have made us overestimate the NCE of the proteins by about 0.5 mEq/100 g protein.

Quite apart from the theoretical difficulties involved, the distribution of the H-ions calculated from the pH measurements in our experiments could not be used for the determination of the Donnan ratio because of a small but systematic error in the determination of the pH of the solutions inside the dialysis units.

found outside a dialysis unit In chapter VII we will nevertheless consider separately the results obtained in the experiments in which (cat min Cl + HCO_3) of the dialysis solution was within the probable range and in those in which (cat min Cl + HCO_3) was clearly outside that range

Apart from the question of their *total concentration* there is the question of *the distribution* of the anions not determined by us It will be evident that Δ (cat min Cl + HCO_3) can only be interpreted as representing NCE if the concentration of all other anions is equal inside and outside the dialysis unit. In view of the effect of the Donnan equilibrium on the one hand and the possible existence of protein bound rest anion on the other hand we will have to be more explicit on this point.

Because of the Donnan equilibrium one would expect the concentration of phosphate (expressed per kg H_2O) to be lower inside the serum-containing unit. However we already discussed in chapter IV the observations of others who found in dialysis or ultrafiltration experiments either an increased concentration at the protein-containing side of the semipermeable membrane (GREENE & POWER 1931 WALSER 1961) or an equal concentration on both sides (HOPKINS *et al* 1952) SEEKLES (1936) who worked with bovine serum, could find no clear evidence of protein bound phosphate Our own results with *in vivo* ultrafiltration agree best with the observations of Hopkins *et al* and indicate that at phosphate concentrations up to 10 mg / and under the conditions of *in vivo* ultrafiltration the effects of binding by protein and of the Donnan equilibrium cancel each other Furthermore we observed in one dialysis experiment with normal dialysis solution no significant difference between the phosphate concentration inside and outside the protein-containing dialysis units (table 36) This table also includes the results of 3 experiments with a dialysis solution containing a fairly high concentration of phosphate buffer instead of the usual bicarbonate/CO_2 mixture The solution contained normal concentrations of Na, K and Cl but no Ca or Mg. It can be seen that at these high concentrations the phosphate content on the inside was slightly lower than on the outside but the difference was not more than 4 %. Walser found that the percentage of inorganic phosphate presumably bound by protein is highest if the phosphate concentration is low We can therefore conclude that at the concentrations used in our experiments there is no significant difference between the concentration of inorganic phosphate inside the serum-containing units and that outside them.



Summary of chapter V

Δ Na, Δ K Δ Ca and Δ Ca + Mg could be determined with reasonable accuracy and expressed in mEq/100 g protein However to obtain from these values the quantity of protein bound cation a correction must be made for the effect of the Donnan equilibrium as the latter causes the concentration of free filtrable cations to be lower on the protein containing side of the membrane than on the other side For this correction the Donnan

ratio must be known. The latter was not determined directly but in chapter VIII an attempt will be made to estimate its value from circumstantial evidence.

The accurate determination of Δ (cat min Cl + HCO_3) is rather difficult in view of the cumulative effect of errors in determination and interpretation. But the available evidence indicates that in the case of the units containing at least 50 g/kg H_2O protein the systematic error – if present – must have been small (less than 1 mEq/100 g protein) and must have led to overestimating rather than underestimating Δ (cat min Cl + HCO_3). The evidence further suggests that the latter is equal to the NCE of the proteins in the experiments in which serum was dialysed. When plasma was dialysed the heparin present may have made us overestimate the NCE of the proteins by about 0.5 mEq/100 g protein.

Quite apart from the theoretical difficulties involved, the distribution of the H-ions calculated from the pH measurements in our experiments could not be used for the determination of the Donnan ratio because of a small but systematic error in the determination of the pH of the solutions inside the dialysis units.

PART III

Results obtained with in vivo ultrafiltration (including normal values for the plasma ions)

Both normal persons and patients have been studied. The results obtained in normal persons will be presented in some detail with due regard to the systematic errors. The results obtained in patients are less accurate since both the number of samples and the percentage increase in protein were smaller than in the normal subjects. But in the case of the patients the results will at least indicate the magnitude of the changes to be expected in the diseases studied.

NORMAL SUBJECTS

DATA CONCERNING SUBJECTS STUDIED AND PRESENTATION OF RESULTS

28 male subjects were studied, all members of our medical staff or senior medical students, varying in age from 23 to 44 years. Some subjects were studied more than once so that the total number of investigations in normal persons was 34. The investigations were done in the course of 1½ year but the conditions of the investigation proper remained constant (see chapter IV).

The result can be described under three headings

(1) the plasma concentrations of Na, K, Ca, Mg, Cl and HCO_3 in the fasting and resting subject (venous blood, obtained without stasis)

(2) values found for 'free' and protein-bound Na, Ca and Mg

(3) values found for RA and for NCE

In table 37 the results of the individual experiments are presented. The values were obtained in the manner described in chapter IV and in table 37 they are not yet corrected for the systematic errors discussed in chapter III and IV. An exception to this are the last columns which give for RA, NCE, 'free' and protein-bound Ca the values corrected for the error resulting from pH change during venous compression. for this *error a* correction can be made in the individual experiment. It should be noted that in table 37 the values for NCE and for protein-bound cation are expressed per 100 g of plasma protein. The actual values in plasma can be calculated with the aid of the protein concentration of the precompression samples

Table 37 further includes data pertinent to the calculation or the interpretation of the results. For each investigation the equation used for calculating water content is given (between brackets the number of samples in which water content was actually determined). Pre- and endcompression pH are listed to indicate the pH change during compression. Protein fractions are given when determined. Furthermore, since both the number of samples and the degree of haemoconcen-

tration affect the accuracy of the regression lines, the total number of samples and the percentage increase in protein concentration ($g/Kg\ H_2O$) are mentioned for each investigation.

In figg. 40a 40b and 40c we have for each experiment graphically represented the data from which NCE and RA were calculated The manner of presentation is the same as in fig 20 (chapter IV) The precompression, compression, and postcompression samples are indicated by different symbols

The graphs will enable the reader to get an idea of the significance of the individual experiments. Obviously less accurate values will be obtained for NCE and RA from experiments with a relatively small number of samples and/or a relatively small increase in protein concentration. Statistically therefore the experiments must have a different 'weight' and this should have its effect on the calculation of the mean value. However in the Addendum it will be shown that the mean values calculated for NCE and for RA with due regard to the differences in the 'weight' of the results of the individual experiments, differ not more than 0.2 mEq/100 g protein, resp 0.2 mEq/Kg H_2O from the mean values calculated by simply averaging the values obtained in the individual experiments. In subsequent calculations we will therefore regard the individual results as having equal 'weight' statistically

We have refrained from graphically reproducing the individual experiments for Na, for Ca and for Ca + Mg both for reasons of space and because for these cations the scatter of the experimental points around the calculated regression line is much smaller than for NCE (VAN LEEUWEN et al 1961).

Since for systematic errors other than those due to the pH change during compression only the direction and the average magnitude can be estimated and their cumulative effect remains relatively small (an exception must be made in the case of protein-bound Na), we will postpone the correction for the systematic errors till after a consideration of some aspects of the experimental results proper

DISCUSSION OF EXPERIMENTAL RESULTS PROPER

Water content of samples – Relation with protein content

As mentioned in chapter IV concentrations were converted from mEq/L plasma to mEq/Kg H_2O in the following manner For at least one pre and one endcompression sample water content was determined (chapter III) On the assumption that the relation between protein content (g/L plasma) and water content (g/L plasma) is linear for the samples of one investigation we calculated for each investigation the constants a and b in the following equation

$$\text{Water (g/L plasma)} = a - b \times \text{protein (g/L plasma)}$$

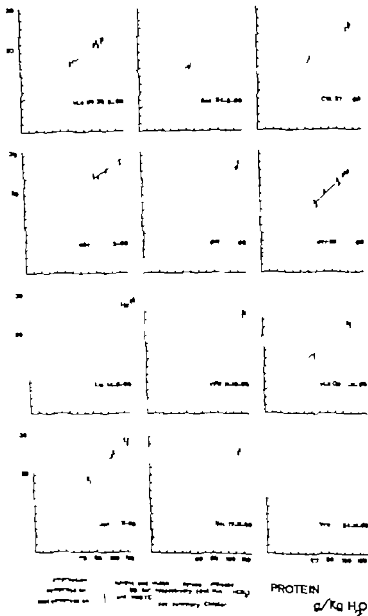
In 31 of the 34 investigations reported in table 37 water content was determined in at least two samples which differed more than 25 g/L in protein concentration In table 37 it can be seen that in these experiments the value found for a varied from 992.5 to 1007.5 and the value found for b from 0 754 to 0 999

Averaging the results for all investigations we found

$$\text{Water (g/L plasma)} = 996.1 - 0.828\ P\ \text{(g/L plasma)}$$

In three cases (Gee dVr I and Dou) the values found for a and b were considerably outside the rather narrow range found for the other experiments. We have no explanation

CATIONS
minus
Cl, HCO_3 mEq/Kg H_2O



PROTEIN
g/Kg H_2O

Fig. 40a. Graphic representation of the 34 *in vivo* ultrafiltration experiments in normal subjects. The diagonal lines represent the regression lines from which NCE and RA were obtained (see table 37).

CATIONS
minus
CL + HCO_3 mEq/Kg H_2O

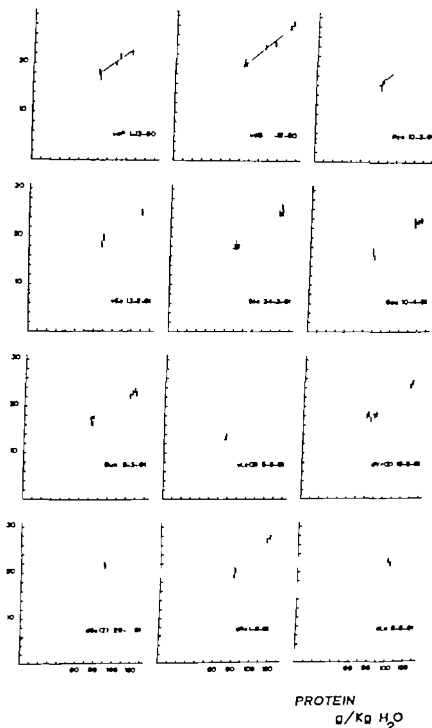


Fig. 40*b* – See caption nd legend of fig. 40*a*.

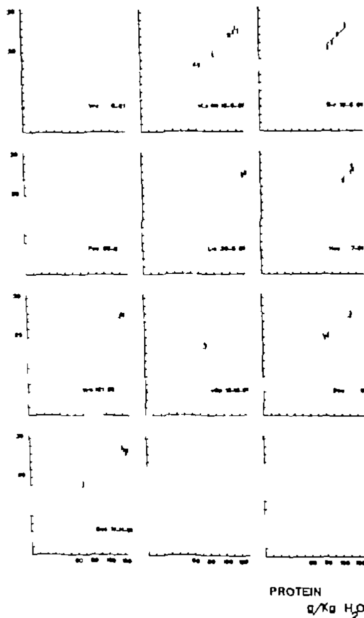


Fig. 40c See caption and legend cf fig. 40a.

for this excentricity the triplicate determinations showed good agreement But if we leave out the three excentric observations we calculate the following average equation for normal plasma

$$W = 995.3 - 0.814 P$$

The equations indicate a specific volume of about 0 82 for the plasma proteins.

In the cases where more than two samples with different protein concentration were dried the experimental points always did lie on a straight line

For each investigation we also calculated the correlation between dry weight and protein concentration (both in g/L plasma) again assuming linearity The dry weight at zero protein concentration represents the weight of all plasma constituents other than protein in as far as they are not bound in one way or other to protein. For all 31 investigations together the average dry weight at protein concentration zero was thus calculated at 9 1 g/L plasma, and when the three 'excentrics' are left out at 9.9 g/L plasma. Since roughly 10 % of Na and of K and about 40 % of Ca and Mg are 'bound' by serum proteins (Gerbrandy et al 1960) we can calculate the dry weight of the remaining cations and anions at 8.5 g/L plasma. In the fasting subject glucose is less than 0.9 g/L and urea about 0.3 g/L. Uric acid, aminoacids and creatinine together should be less than 1 g/L. The lipids present in plasma from a fasting subject are completely bound to proteins and will not contribute to the dry weight at zero protein concentrations. There is therefore a reasonable agreement between the expected dry weight and the value found by extrapolation from our experimental values.

In the 18 dialysis experiments performed with serum of normal subjects (practically all of whom also partook in the *in vivo* ultrafiltration study) we found the following average equation

$$\text{Water (g/L plasma)} = 996.0 - 0.815 P \text{ (g/L plasma)}$$

In the individual experiments the value of a varied between 995 5 and 997 7 and that of b between 0 763 and 0 855 In these experiments the relation was calculated from the dry weight of the dialysis solution and that of one or more of the serum samples.

We have preferred to use the correlation found in the individual experiment instead of the average equation for calculating concentrations per Kg H_2O because in the case of patients only the individual correlation can be used some nephrotics have a rather high specific volume of the proteins presumably because of the secondary hypercholesterolaemia observed in these cases In the uraemics the value of the extrapolated dry weight was occasionally as high as 16 g/L.

Although the use of the individual correlation instead of the average equation must somewhat increase the spread in the values obtained for protein bound cation the effect is negligible in the case of NCE since both cations and anions are multiplied with the same factor Even in the three cases where the correlation between water content and protein concentration was well outside the range observed in the other normal subjects, the values of NCE obtained from molal concentrations calculated either by means of the individual correlation or by means of the average equation found for normal subjects differed less than 0.1 mEq/100 g protein In the three *in vivo* experiments and two dialysis experiments in which no water content was determined the general equation 996–0 82 P was used.

A comparison of our values for plasma water with those found by others must wait till another occasion Also for reasons of space, we shall not discuss the water content found in the case of the patients or in the case of the dialysis experiments.

The correlation between (cat min Cl + HCO_3) and protein concentration as observed in the individual experiments

For this we must consider the experiments in which the protein concentration of the various compression samples shows a gradual rise resulting in a uniform distribution of the experimental points along the regression line (figs. 40*a* 40*b* 40*c*). In most of these experiments the linearity is evident but in some (dVr 18–5–61 Poo 28–6–61 and vOp 16–10–61) the points representing the first compression samples clearly fall below the regression line.

This dip was not seen in the correlation between Na, Ca or Ca + Mg and protein concentration. In the three experiments mentioned above the low value for (cat min Cl + HCO_3) in the first compression samples resulted from the fact that the HCO_3 concentration increased immediately after starting compression whereas the Cl concentration decreased more gradually. We have not been able to explain this phenomenon. A relative increase in γ-globulin which would decrease NCE was never observed nor sudden decrease in pH, in lactate or in inorganic phosphate concentration.

But even if this deviation from linearity is real its effect on the mean values calculated for NCE and RA in normal subjects must have been small. This can be demonstrated by calculating, for each of the experiments in which the points representing the compression samples are distributed in a uniform manner over the protein range, the regression line for the compression points only. The slope of the regression line thus calculated will largely depend on the points representing the first samples taken after the start of compression, since they determine the position at its lower end. A slight downward deviation of these points will cause the slope of the regression line calculated for the compression points to be much steeper than that of the regression line calculated for all points. Table 38 demonstrates that there is indeed a difference between the mean values of NCE and of RA calculated from all samples of an experiment (first 2 columns), and the values calculated from the compression samples only (last 2 columns), but that the difference is a small one. Furthermore since the number of points available for the calculation of the second regression line is rather small, especially at the critical lower end of the line, the accuracy of the values in the last 2 columns is less than that of the values in the first 2 columns and we have therefore concluded that in our experiments the correlation between (cat min Cl + HCO_3) and protein concentration is on the average sufficiently linear to allow the calculation of one regression line for all the samples of an experiment. The following observations appear to endorse this conclusion. In 5 experiments the first postcompression sample was taken within two minutes after release of the pressure and therefore still has a rather high protein concentration. The value found for (cat min Cl – HCO_3) in these samples fits remarkably well into the values found for the compression samples. There is thus no sign of hysteresis and a similar conclusion can be drawn from the finding that pre and postcompression samples show on the average nearly the same values for (cat min Cl + HCO_3). (See table 27 in chapter IV)

Correlation between RA and NCE and between 'free' cation and protein-bound cation

Theoretically no correlation should exist between the values calculated for RA and for NCE, whereas one would expect a positive correlation between the concentration of 'free' cation and the amount of protein-bound cation. In fig. 41 we have plotted the

values given in table 37 for NCE respectively protein bound cation against the corresponding values for RA respectively free cation (the values are those not corrected for any systematic error) It will be seen that in every instance a negative correlation was found The obvious explanation is that RA respectively free cation concentration is not determined independently but is obtained by extrapolating the regression line to protein concentration zero The normal scatter in the slope of the calculated regression line will therefore result in an inverse scatter of the values found for RA respectively free cation From fig. 41 it is evident that within the normal range this scatter effect overrules the fundamental physico-chemical relationship This observation serves to stress the fact that for RA and for free cation the average of the individual values rather than the individual values themselves should be considered

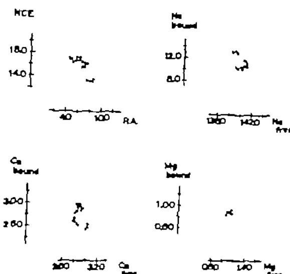


Fig. 41 – Correlation between RA and NCE, respectively 'free' and protein-bound cation as observed in the normal subjects (RA and 'free' cation in mEq/Kg H_2O NCE and protein-bound cation in mEq/100 g protein).

Changes in normal values observed in the course of one and a half year

In fig. 42 the values given in table 37 for plasma concentration and for NCE and protein bound cation (not corrected for any systematic error) have been plotted against the date of the investigation It can be seen that the values show a reasonably equal distribution around the lines representing the mean values calculated from the first 28 experiments in table 37 Fig. 42 also demonstrates that the values found on repeated investigation of the same subject show a spread similar to that found for the group of normal subjects considered as a whole at least for NCE and protein-bound cation

CORRECTION FOR SYSTEMATIC ERRORS

In chapter III and IV we discussed a number of systematic errors for which the results given in table 37 should be corrected. For all but one of the errors only the average effect can be estimated and this with a varying degree of certainty In the following paragraphs the magnitude and direction of these errors are considered (for argumentation the reader is referred to chapter III and IV).

The magnitude of the four errors to be considered first can be estimated with a fair degree of certainty but in the case of the last two the estimate is much more dubious

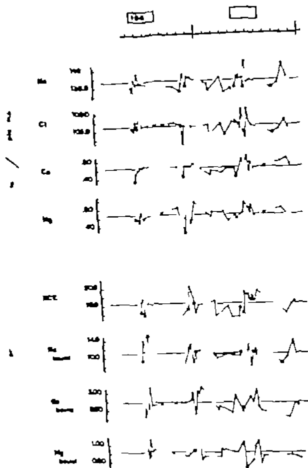


Fig. 42 Relation between date of experiments and values for normal subjects The symbols o, x, Δ and □ indicate the four persons who were studied more than once.

(a) *Effect of the heparin solution added* This will depend on the amount and the composition of the heparin solution added and on the plasma volume of the blood sample. For the samples of one experiment the first are constant but the latter varied according to haematocrit and total volume of the blood sample Since the haematocrit of both pre- and an endcompression sample was determined in 17 of the experiments in table 37 (numbers 1 16 and 29), we can use the results of these experiments for calculating the magnitude of the heparin error in the following manner When the amount of heparin solution (V_{hep}) and its composition (C_{hep}) are know as well as the combined volume of the plasma-heparin mixture (V_{pl+hep}) and its composition (C_{pl+hep}) the concentration in the original plasma (C_{pl}) can be calculated from

$$C_{pl} \quad V_{pl} = (C_{pl+hep} \quad V_{pl+hep}) - (C_{hep} \quad V_{hep}).$$

I the 17 experiments, referred to above, the average value of the haematocrit was 44 for the precompression and 57 for the endcompression samples whereas the sample volumes were on the average 16 and 17 ml respectively The composition of the heparin solution is known (see chapter III) and with the aid of these data and of the equation mentioned bove we could make an estimate of the resulting errors.

I the case of protein, Ca, Mg and HCO_3 the concentration decreases by 0 1 respectively 0.3 as result of adding 0 01 respectively 0 03 ml heparin solution. These errors are so small, both

absolute and relative, that we have neglected them. Since the Cl and K concentration of the heparin solution are of the order of the plasma concentration of these ions no error is made in their case. For Na the concentration increases by about 0.1 respectively 0.3 / when 0.01 respectively 0.03 ml heparin solution are added. In this case the absolute error is not negligible and owing to the fact that the error was somewhat larger in the end compression samples (where the plasma volume was smaller than in the precompression samples) protein-bound Na is also somewhat overestimated. As a result NCE and RA are overestimated as well. In table 39 the magnitude of these errors is shown (the error given for protein-bound Na and for NCE includes the error due to dilution of the protein concentration).

As during the first part of our study 0.01 ml heparin solution was added to the samples but later 0.03 ml the mean values of the corresponding episodes were corrected separately.

(b) *Flame photometer error in determination of Na and K.* In chapter III we concluded that both Na and K concentrations are underestimated by on the average 0.1 mEq/L at a protein concentration of 60 g/L and 0.2 mEq/L at a protein concentration of 120 g/L. The values obtained on precompression samples are presumably therefore for both cations on the average 0.1 mEq/L too low. Furthermore, as a result of the extrapolation the amount of protein-bound Na will be underestimated by $100/60 \times 0.1 = 0.15$ mEq per 100 g protein, and NCE (to which both Na and K contribute) by double this amount i.e. 0.3 mEq per 100 g protein. The respective average values must be corrected accordingly. The error has no influence on the calculation of the amount of 'free' Na and K and of RA.

(c) *Error caused by change in pH during compression of upper arm.* As both the pH and the protein changes were determined in each experiment it is possible to correct the individual results in the case of NCE and of protein-bound Ca, because data exist concerning the change of these quantities with pH (chapter IV). The corrected values thus obtained are listed in the last columns of table 37. For protein-bound Na and Mg only an approximate correction of the average values can be made.

(d) *Error caused by measuring blood pH at 37.5° C.* As stated in chapter IV the temperature of the venous blood must have been about 35.5° C on the average. The resulting error in pH measurement – an underestimate of 0.03 – causes HCO_3 to be underestimated by 0.1 mEq/Kg H_2O at the most. But this only influences the calculation of RA, the average value of which should accordingly be corrected by subtracting 0.1 mEq/Kg H_2O. The pH to which the values calculated for protein-bound cation and for NCE must be referred is the average value of the endcompression pH (see chapter IV) with 0.03 added for the temperature error. Similarly to obtain the pH of venous plasma in the fasting and resting subject, 0.03 must be added to the average precompression pH value.

(e) *Error due to neglect of the presence of carbonate and of carbamino-protein.* In chapter III reasons were given for assuming the combined effect to be an overestimate of HCO_3 by 0.1 mEq/L at pH 7.40 and by 0.2 mEq/L at pH 7.80 in otherwise normal plasma. Because of its linear correlation with protein concentration the error due to carbamino-protein was estimated to increase from + 0.2 to + 0.4 mEq/L, if the protein concentration increases from 60 to 120 g/L. Accordingly NCE will be underestimated by $100/60 \times 0.2 = 0.3$ mEq/100 g protein and the value of RA will be overestimated by 0.1 mEq/Kg H_2O. These values should apply when pH is about 7.40.

(f) *Error due to the effect of the Donnan equilibrium.* In chapter IV it was concluded that the calculation of NCE is not affected by this error but that the calculation of protein-bound Na, Ca and Mg is. In chapter IV we have estimated the error to be an underestimate of 3–4 mEq/100 g protein in the case of Na, and an underestimate of 0.1–0.2 mEq/100 g protein in the case of Ca. Since in the case of Mg about the same percentage of the total cation concentration is protein-bound as in the case of Ca, and presumably in the same manner, it is reasonable to assume the same percentage underestimate of protein-bound Mg i.e. about 0.05 mEq/100 g protein. Since the error due to the Donnan equilibrium is linearly related to the protein concentration it has no effect on the calculation of the free fractions at zero protein concentration. But at the actual protein concentration of the precompression samples the concentration of 'free' cation must have been about 3 mEq/Kg H_2O lower for Na and about 0.1 mEq/Kg H_2O lower for Ca.

In the following tables the experimental values given in table 37 are corrected for the errors mentioned and a best estimate of the average values is made.

Table 40 summarises *the* (*venous*) *plasma concentration* (mEq/L) *of Na K, Ca Mg Cl and* HCO_3 *in the fasting and resting subject* (*blood obtained without stasis*). In this table also the *protein concentration* and *composition* are given and the concentration found for *inorganic phosphate*

In table 41 *the values for free* (mEq/Kg H_2O) *respectively protein-bound* (mEq/100 g protein) *Na, Ca and Mg are given.*

Table 42 finally presents *the values for RA* (mEq/Kg H_2O) *and for NCE* (mEq/100 g protein).

It is evident that the total error for RA and for NCE is largely determined by the error caused by the change in pH during venous compression

ADDITIONAL OBSERVATIONS

Correlation between endcompression pH respectively protein composition and protein-bound cation and NCE

As both pH and protein composition affect the amount of cation bound by protein it is of some interest to see whether this effect is detectable within the range of normal values. To that purpose fig. 43 was constructed.

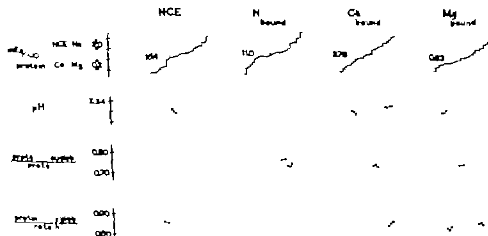


Fig 43 Correlation between endcompression pH, respectively protein composition, and NCE and protein-bound cation (normal subjects)

We have arranged the individual values presented in table 37 according to the magnitude of NCE respectively protein-bound cation, starting with the lowest value. Below these values the concomitant endcompression pH and protein composition were plotted. The latter is indicated by the ratio (protein − α-glob)/protein respectively the ratio (protein − γ-glob)/protein according to whether the method of Majoor was used or paper electrophoresis. We have chosen these ratios because the evidence available at present suggests that albumin, α-globulin and β-globulin have the highest cation-binding capacity and γ-globulin the lowest.

Fig 43 shows that only between protein bound Ca and protein composition some correlation appears to exist Furthermore we found that in the case of NCE and of protein bound Ca it makes no difference whether the experimental values proper or the values corrected for the effect of pH change are considered The correction does not affect the spread nor the distribution of the individual values around the mean value

Comparison of the values obtained for protein-bound Ca and Mg with the values obtained in earlier experiments

We have previously published values for 'free and protein bound Ca and Mg obtained with the same method (VAN LEEUWEN *et al* 1961) In table 43 the results of the earlier study are compared with the present results. Since in the first study no correction was made for systematic error the results of the present study are also given without correction The two series have only one experiment in common (Lie 14-6-60) but 12 of the subjects figure in both series From table 43 it would appear that the values found for protein-bound cation in the present study are about 6 / lower than those found in our previous study The difference is due to the fact that the protein concentration obtained in the first study was afterwards found to be 3 / too low up to a protein concentration of about 70 g/L the error became increasingly larger for protein concentrations higher than 70 g/L. The error was due both to an error in the Kjeldahl determination and to an error in the calibration line used with the biuret method (see chapter III) Because of this the amount of cation bound per 100 g protein was in the earlier experiments overestimated by about 5 / and – as a result of extrapolation – the concentration of free cation was underestimated by about 2 /. This fully explains the difference between the results of the two studies

Results obtained when Na and K are analysed in deproteinised plasma

In a number of experiments presented in table 37 Na and K were determined both in plasma and in deproteinised plasma since at the time we could not yet decide which of the two techniques was the more accurate one However during that period 6 investigations on patients were done in which only deproteinised plasma was analysed These observations cannot be compared with the results obtained for the other patients unless a correction is made To provide the latter table 44 has been added which shows the values obtained when plasma and the values obtained when deproteinised plasma was analysed in 14 experiments with normal subjects As the difference between the Na concentration obtained with plasma and that obtained with deproteinised plasma increases with increasing protein concentration (see chapter III) not only a higher value for RA is found with deproteinised plasma but also a higher value for NCE. On the average the difference was 1 3 mEq/Kg H_2O for RA and 1 9 mEq/100 g protein for NCE.

PATIENTS

DATA CONCERNING SUBJECTS STUDIED AND PRESENTATION OF RESULTS

29 patients of both sexes (age 25 to 87 yr) were studied in the manner described in chapter IV The diseases from which they suffered can be classified in two groups

(1) Diseases mainly characterised by an *abnormality in protein composition* either a

quantitative disproportion between the normal fractions (which shall be indicated by the term dysproteinaemia) or the presence of an abnormal protein (indicated as paraproteinaemia).

(2) Diseases mainly characterised by an *abnormality in the other plasma constituents*. The patients with severe chronic uraemia are grouped together. The others – including patients with hypercalcaemia and hypercholesterolaemia – are listed under miscellaneous together with one apparently normal reconvalescent.

The conditions of the actual investigation were the same as for the normal persons, but in 6 patients Na and K concentration were determined on deproteinised plasma only.

The results of the individual experiments are compiled in table 45. The lay-out of the table differs somewhat from that of table 37 and needs some explanation.

The results have been grouped according to the main abnormality in the plasma but overlapping could not be avoided.

In table 45 the Hb concentration has also been listed. In the presence of severe anaemia venous congestion tends to raise venous blood lactate (chapter IV) and neglect of this would result in the calculation of too high a value for NCE and too low a value for RA. Consequently special caution is necessary in the interpretation of these values when Hb is low.

When in the column headed 'other substances' creatinine is not mentioned the glomerular filtration was at least 70 ml/min.

In the last four columns are listed the values found for RA, NCE, 'free' and protein-bound Ca after correction for the error caused by the pH change during compression. In the case of the patients this correction was sometimes rather large because, although the pH change was of the same order as in the normal subjects, the percentage rise in protein concentration was considerably smaller in most patients.

At the end of each subgroup we have listed the results of the investigations in which Na and K were only determined on deproteinised plasma. Both the plasma concentration of these substances and the values calculated for RA and NCE, respectively 'free' and protein-bound Na are too high. These values have therefore been marked with an asterisk.

For normal subjects the results obtained with deproteinised plasma are on the average 1.3 higher for RA (mEq/Kg H_2O) and 1.9 higher for NCE (mEq/100 g protein) (table 44). But for the 6 investigations of patients, in which flame photometry was performed both on plasma and on deproteinised plasma, the values calculated from the latter were on the average 2.1 higher for RA and 1.0 higher for NCE (table 46). The difference between the average values in table 44 and the corresponding average in table 46 is presumably due to the fact that the protein concentration was less in the patients. We have therefore concluded that the values which would have been obtained if plasma had been analysed, can be estimated by subtracting from the values actually obtained with deproteinised plasma 1.5 for RA (mEq/Kg H_2O) and 1.5 for NCE (mEq/100 g protein). This correction should increase the inaccuracy of the individual results by not more than 2 mEq for either RA or NCE (see table 44 and 46).

COMPARISON WITH THE RESULTS OBTAINED IN NORMAL SUBJECTS

NCE and RA

In fig. 44 the results obtained in patients are compared with the values found for normal subjects. Disease can alter the protein concentration of plasma, the protein composition or both. We have therefore compared the actual values of NCE (mEq/L plasma) as well as the values obtained when NCE is expressed per unit weight of protein (mEq/100 g). The first values indicate the effect of the disease on the NCE of plasma, the latter give information concerning changes in the characteristics of the proteins themselves.

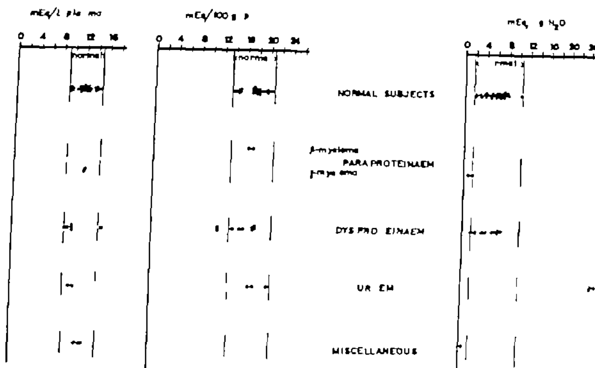


Fig. 44 – Comparison of results obtained for patients with those obtained for normal subjects. For explanation see text.

All points in fig. 44 refer to the values obtained after correction for the effect of pH change during venous compression. The values from experiments in which Na and K were determined on deproteinised plasma are indicated by open circles. They were obtained by subtracting 1.5 from those values listed for RA and for NCE in the last four columns of table 45 which are indicated by an asterisk. Finally the actual plasma values for NCE were calculated from the (corrected) values per 100 g protein and the protein concentration of the precompression samples (also listed in table 45).

Somewhat to our surprise we found NCE in the normal range in most of the patients we studied, even in those who were severely uraemic. Indeed, if we take into account the larger spread to be expected in the case of the patients, we must conclude that *only* in the patients with paraproteinaemia abnormal values were found. For the patients with γ myeloma the values are definitely *too low* when expressed per *100 g protein* but when expressed per litre plasma they are nearly normal because of the high protein concentration in plasma. *On the other hand in the 2 patients with β-myeloma* NCE expressed per 100 g protein was normal, but expressed per litre plasma NCE was somewhat *too high, again because of the increased total protein concentration*. Evidently the paraproteins of the γ-globulin type have a very low NCE whereas those of the β-type *appear to have a NCE comparable to that of albumin*. The results, obtained in the case of γ myeloma, are in agreement with the findings of Gutman *et al* discussed in chapter II.

For RA abnormal values were only found in the case of the uraemic patients. In this

group there is a positive correlation between the value found for RA and the concentration of inorganic phosphate, the latter accounting for about 30 / of the former (table 45). The same ratio was found in normal subjects but whether this observation has any meaning cannot be decided on this small number of investigations.

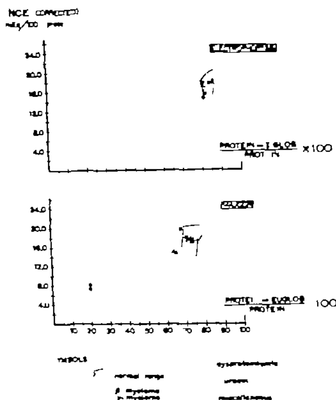


Fig 45 Correlation between composition of plasma protein and NCE (patients)

Effect of protein composition on NCE An attempt to correlate NCE (expressed in mEq/100 g protein) with protein composition meets with the difficulty that there are more than two protein fractions. And since we analysed plasma, fibrinogen was also present, which in the patients occasionally constituted as much as 20 / of total protein. But the following reasoning appears to provide a means for analysing the effect of protein composition. From the dialysis experiments to be discussed in the next chapter we must conclude that both for normal and for abnormal γ-globulin NCE is practically zero at normal plasma pH Consequently NCE should show a positive correlation with (total protein γ-globulin) expressed as percentage of total protein. Fig. 45 appears to confirm this but the number of observations with a low value for (total protein – γ-globulin) is ery small and fig 45 does no more than indicate a certain trend.

As regards the relative contributions of albumin and of α and β-globulin and fibrinogen to the value of NCE the following conclusions can be drawn from table 45 In the group of dysproteinaemias there are 2 patients with a low albumin and a low γ

globulin content (Ben and Ber) and in these cases NCE was therefore largely determined by the other protein fractions in both cases a normal value for NCE was found. This suggests that for α β-globulin and fibrinogen together NCE (in mEq/100 g protein) is of the same order as for albumin. Furthermore, for the patients with dysproteinaemia the average value of NCE was of the same magnitude as for the patients with uraemia and for the patients listed under miscellaneous Since in the dysproteinaemia group the percentage albumin was on the average about half that found in the two other groups whereas the percentage γ-globulin was higher we are tempted to postulate that – per unit weight – α β-globulin and fibrinogen together have a higher NCE than albumin

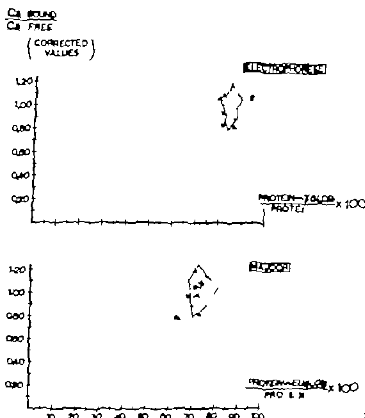


Fig. 46 Correlation between composition of plasma protein and the ratio protein-bound Ca/free Ca (patients) See text for explanation

Effect of pH on NCE Until now we have neglected the influence of the pH Differences in the latter must have contributed to the spread in the values obtained The extremes in endcompression pH observed in the patients were 0.25 pH unit apart and therefore purely because of the pH effect, the value of NCE should for these two patients differ by about 3 mEq/100 g protein if we accept the buffervalue found by VAN SLYKE *et al* in 1928 However in most investigations the endcompression pH was in the same range as that found for the normal subjects. The 4 groups of patients differ somewhat in this respect the average endcompression pH being slightly higher for the patients with paraproteinaemia and somewhat lower for the patients with uraemia. This in itself should cause NCE to be overestimated in the first group and to be underestimated in the uraemic patients, but the difference between the pH values is so small that a corresponding correction of the average NCE values did not visibly change the relation between the 4 groups depicted in figg 44 and 45

Free and protein-bound Na, Ca and Mg

For all three cations the amount bound per 100 g protein is lowest in the patients with paraproteinaemia and in the case of Na the difference between the β- and the γ-type of myeloma is evident. The difference is not so evident for Ca and in the case of Mg no difference can be detected. But especially for Ca the 'free' concentration varied considerably more in the patients than in the normal subjects and for this a correction should be made. To that purpose we have calculated for each experiment the ratio between protein-bound Ca (mEq/100 g protein) and 'free' Ca (mEq/Kg H_2O), using the values obtained after correction for the error caused by the pH change during compression. This ratio should be constant if no change occurs in protein composition or in pH (McLean & Hastings 1935[1]). In fig. 46 we have plotted this ratio against the ratio (total protein − γ-globulin)/total protein, and it can be seen that roughly the same correlation exists as for NCE: the abnormal γ-globulins bind considerably less Ca than the other fractions, but albumin and the other globulins apparently have about the same tendency to bind Ca, at least at normal pH values.

For Mg a similar attempt would be wasted because the spread in individual values is too large in comparison with the correlation to be detected. And since in the case of protein-bound Na the correction for the effect of the Donnan equilibrium is about 30 % of the uncorrected value we have also refrained from considering the correlation between protein composition and protein-bound Na in more detail.

Results obtained with equilibrium dialysis

Most experiments were done with serum, a smaller number with solutions of albumin and γ globulin. The few experiments in which plasma was dialysed will be presented together with the results obtained with serum of normal subjects

SERUM FROM NORMAL SUBJECTS

DATA CONCERNING SUBJECTS STUDIED – PRESENTATION OF RESULTS

23 dialysis experiments were done between November 1960 and February 1962. In 5 experiments plasma was dialysed, in 17 serum and in 1 experiment both plasma and serum In 19 experiments the serum or plasma was obtained from one (fasting) subject but in 4 experiments pooled serum was studied each sample containing serum from 3 (non-fasting) subjects. The samples were dialysed directly after they were obtained Since some subjects contributed more than once the total number of persons whose serum or plasma was studied is 19 one a female. All but 3 also underwent *in vivo* ultrafiltration and in most cases the sample used for dialysis was obtained immediately after the conclusion of the *in vivo* ultrafiltration experiment.

Nearly all serum samples were dialysed at three different pH values. The latter ranged from 6.33 to 7 96 but in all but 3 of the experiments the pH was within the range 6 60 to 7 80

For the description of the method and the calculations involved the reader is referred to chapter V

The results of the individual experiments (no correction for systematic errors) are given in table 47 For reasons of economy the small number of experiments in which pathological serum was dialysed have been listed at the end of table 47 They will be discussed separately Table 47 needs some explanation

The heading distilled water' refers to the solution inside the control unit after dialysis, the heading dialysis solution to the composition of the solution outside the dialysis units at the end of the dialysis. Under the headi g protein solution are indicated respectively the number of dialysis units filled from the same serum (plasma) sample whether the protein solution was plasma or serum the mean pH value, the mean protein concentration and the mea values for NCE, Δ Na, Δ K and Δ Ca + Mg for the number of units indicated (the maximum difference in protein concentration between the units filled from one sample was never more than 7 g per Kg H_2O) For reasons discussed in chapter V only the results from units containing solution with more than 50 g/Kg H_2O protein have been included in table 47 As to the protein composition the values placed between brackets are the values found for *plasma* in the preceding *in* *vivo* ultrafil-

tration experiment. In most cases serum was dialysed but the relative percentages of albumin and the different globulins must have been practically the same as for plasma.

Finally in the columns containing the data concerning the concentration ratios the heading distilled water refers to the distribution across the membrane of the control unit, the heading protein solution to the distribution across the membrane of the serum or plasma containing units. In the latter case all ratios refer to protein concentration of 70 g/Kg H_2O the reason for this will be discussed in the next section.

For Ca and Mg no separate results are given. In order to reduce the amount of analytical work only the sum concentration Ca + Mg was determined in all experiments but 4 and in these plasma was studied. The values obtained for Δ Ca and Δ Mg in these 4 experiments will be presented separately

Concerning the interpretation of changes in Ca + Mg the following can be said. Both CARR (1955) and VAN OS & KOOPMAN – VAN EUPEN (1957) observed that albumin attracts Ca and Mg-ions apparently with equal strength. Presumably therefore a given change in pH will induce the same relative changes in albumin-bound Ca and in albumin-bound Mg. Although in serum the proteins bind a higher percentage of Ca than of Mg the difference is not large (see chapter VI) and in our dialysis experiments we can presumably interpret changes in the serum concentration of Ca + Mg as indicating equal (relative) changes in the concentration of each of the two cations.

SOME POINTS CONCERNING EXPERIMENTAL RESULTS AND THEIR PRESENTATION

Correlation between the values found for (cat min Cl + HCO_3) in the dialysis solution and the values calculated for Δ (cat min Cl + HCO_3) i.e. NCE

In chapter V it was pointed out that in a fairly large number of experiments the value found for (cat min Cl + HCO_3) in the dialysis solution was outside the range expected

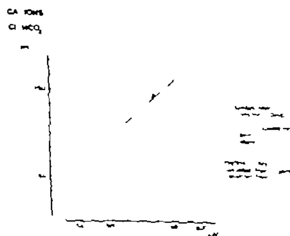


Fig 47 Relation between the values found for (cat min Cl + HCO_3) in the dialysed solution and the values obtained for Δ (cat min Cl + HCO_3). See text.

from the known concentration of the other *filtrable anions* present in serum. Reasons were given why we assume that this *unexplained discrepancy* does not invalidate the

Results obtained with equilibrium dialysis

Most experiments were done with serum a smaller number with solutions of albumin and γ globulin The few experiments in which plasma was dialysed will be presented together with the results obtained with serum of normal subjects

SERUM FROM NORMAL SUBJECTS

DATA CONCERNING SUBJECTS STUDIED – PRESENTATION OF RESULTS

23 dialysis experiments were done between November 1960 and February 1962. In 5 experiments plasma was dialysed in 17 serum and in 1 experiment both plasma and serum *In 19 experiments the serum or plasma was obtained from one (fasting)* subject but in 4 experiments pooled serum was studied each sample containing serum from 3 (non fasting) subjects The samples were dialysed directly after they were obtained. *Since some subjects contributed more than once the total number of persons* whose serum or plasma was studied is 19 one a female All but 3 also underwent *in vivo* ultrafiltration and in most cases the sample used for dialysis was obtained immediately after the conclusion of the *in vivo* ultrafiltration experiment

Nearly all serum samples were dialysed at three different pH values. The latter ranged from 6 33 to 7 96 but in all but 3 of the experiments the pH was within the range 6.60 to 7 80

For the description of the method and the calculations involved the reader is referred to chapter V

The results of the individual experiments (no correction for systematic errors) are given in table 47 For reasons of economy the small number of experiments in which pathological serum was dialysed have been listed at the end of table 47 They will be discussed separately Table 47 needs some explanation

The heading distilled water refers to the solution inside the control unit after dialysis, the heading *dialysis solution* to the *composition of the solution outside the dialysis* units at the end of the dialysis. Under the heading protein solution are indicated respectively the number of dialysis units filled from the same serum (plasma) sample whether the protein solution was plasma or serum the mean pH value, the mean protein concentration and the mean values for NCE, Δ Na, Δ K and Δ Ca + *Mg for the number of units indicated (the maximum difference in protein* concentration between the units filled from one sample was never more than 7 g per Kg H_2O). For reasons discussed in chapter V only the results from units containing a solution with more than 50 g/Kg H_2O protein have been included in table 47 As to the protein composition the values placed between brackets are the values found for *plasma* in the preceding *in vivo* ultrafil-

tration experiments. In most cases serum was dialysed but the relative percentages of albumin and the different globulins must have been practically the same as for plasma.

Finally in the columns containing the data concerning the concentration ratios the heading 'distilled water' refers to the distribution across the membrane of the control unit, the heading 'protein solution' to the distribution across the membrane of the serum or plasma containing units. In the latter case all ratios refer to a protein concentration of 70 g/Kg H_2O; the reason for this will be discussed in the next section.

For Ca and Mg no separate results are given. In order to reduce the amount of analytical work only the sum concentration Ca + Mg was determined in all experiments but 4 and in these plasma was studied. The values obtained for Δ Ca and Δ Mg in these 4 experiments will be presented separately.

Concerning the interpretation of changes in Ca + Mg the following can be said. Both CARR (1955) and VAN OS & KOOPMAN – VAN EUPEN (1957) observed that albumin attracts Ca- and Mg-ions apparently with equal strength. Presumably therefore a given change in pH will induce the same relative changes in albumin-bound Ca and in albumin bound Mg. Although in serum the proteins bind a higher percentage of Ca than of Mg the difference is not large (see chapter VI) and in our dialysis experiments we can presumably interpret changes in the serum concentration of Ca + Mg as indicating equal (relative) changes in the concentration of each of the two cations.

SOME POINTS CONCERNING EXPERIMENTAL RESULTS AND THEIR PRESENTATION

Correlation between the values found for (cat min Cl + HCO_3) in the dialysis solution and the values calculated for Δ (cat min Cl + HCO_3) i.e. NCE

In chapter V it was pointed out that in a fairly large number of experiments the value found for (cat min Cl + HCO_3) in the dialysis solution was outside the range expected

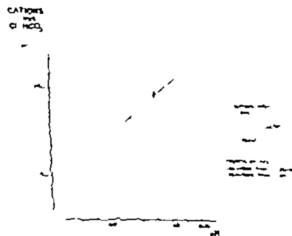


Fig. 47. Relation between the values found for (cat min Cl + HCO_3) in the dialysis solution and the values obtained for Δ (cat min Cl + HCO_3). See text.

from the known concentration of the other filtrable anions present in serum. Reasons were given why we assume that this unexplained discrepancy does not invalidate the

determination of NCE. We are *now in a position to test this* assumption in the following manner In fig. 47 all values obtained for Δ (cat min Cl + HCO_3) with normal serum or plasma are plotted against pH *using different symbols to indicate whether the value* actually found for (cat min Cl + HCO_3) in the dialysis solution was higher than expected, as expected or lower than expected It will be *seen that the three groups of symbols* intermingle completely Exclusion of all experiments in which the value of (cat min Cl + HCO_3) in dialysis solution was outside the probable range – *i.e.* differed by more than 2 SD from the expected value – has virtually no influence on slope and position of the regression line calculated for Δ (cat min Cl + HCO_3) on pH

The regression lines were calculated by the method of least squares and they only serve as a means for comparing the two *groups of experimental points. It is not suggested that they indicate* the true relationship between pH and NCE, which will be discussed later

Necessity of expressing concentration ratios at a uniform protein concentration

The concentration ratio of a particular ion depends on the concentration of its 'free' fraction and on protein net charge The first has been fairly constant in our experiments, but the second differed considerably as a result of differences both in total protein concentration and in pH (for the normal subjects protein composition was sufficiently similar to be considered constant) If we want to study the effect of changes in pH on the concentration *ratios* the effect of a change in protein concentration *has to be* eliminated by calculating the ratios at a uniform protein concentration For the latter we *have chosen the value of 70 g/Kg H_2O At this protein concentration the concentration* ratios will be comparable with the values published previously for the concentration *ratios at the boundary serum – interstitial fluid*

The ratio to be expected at a protein concentration of 70 g/Kg H_2O was obtained in the following manner (Na is taken for an example) from $[Na]_i$ $[Na]_o$ and the actual protein concentration Δ Na is calculated and expressed per 70 g protein. By adding this value to the concentration found in the dialysis solution we can calculate both the Na concentration inside and the concentration ratio to be expected at a protein content of 70 g/Kg H_2O For the anions the concentration inside decreases with increasing protein concentration, but otherwise the procedure is the same *Its* justification is to be found *in* chapter V where it was shown that over the range 15 to 70 g/Kg H_2O protein the Δ values are linearly related to the protein concentration *inside* the unit with the possible exception of Δ Cl and Δ (cat min Cl + HCO_3) But for the protein range, with which we are here concerned, *i.e. 52 to 74 g/Kg H_2O* the linearity of the relation seems well established even for Δ Cl.

We can therefore write

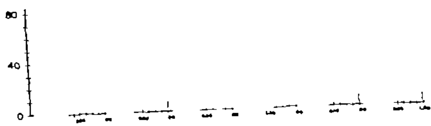
$$[Cat]_i = [Cat]_o + 0.01\ \Delta\ Cat\ [Pr]_i$$

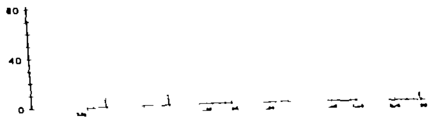
where Δ Cat stands *for* Δ Cat expressed per 100 g protein and $[Pr]_i$ *for the actual protein concentration* inside the dialysis unit. The concentration *ratio* is then

$$R_{ca} = \frac{[Cat]_o}{[Cat]_i} = \frac{[Cat]_o}{[Cat]_o + 0.01\ \Delta\ Cat\ [Pr]_i}$$

Similarly we find

$$R_{an} = \frac{[An]_i}{[An]_o} = \frac{[An]_o - 0.01\ \Delta\ An\ [Pr]_i}{[An]_o}$$





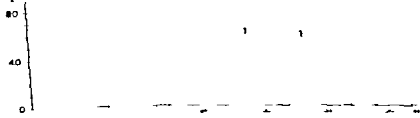


Fig. 48. Relation between protein concentration and concentration ratios of Na, Cl and Ca + Mg. The diagonal lines have no statistical meaning but simply connect the average concentration ratio found at the highest protein concentration with the point representing concentration ratio 1.00 at protein concentration zero.

As long as the concentration of $[Cat]_0$ is large in comparison to Δ Cat *(as in the case of* Na and K) the relation between concentration ratio and protein concentration is practically linear for protein concentrations up to 100 g/Kg H_2O But if Δ Cat is of the same magnitude as $[Cat]_0$ – as in the case of Ca and Mg – the relation is curvilinear

In chapter I a similar relation was deduced for the Donnan ratio and fig. 48 shows for Na, Cl and Ca + Mg the relation experimentally found in the experiments in which various dilutions of the serum were dialysed in one container It can be seen that for Na and Cl the relationship is indeed rectilinear within the error of determination, whereas for Ca + Mg the curvilinearity is evident K and HCO_3 are not shown because of the much larger spread in their case – but the position of the points was found compatible with rectilinearity

From fig 48 the necessity of expressing concentration ratios at uniform protein concentration should be evident, and since in the experiments represented in table 47 the actual protein concentration was always higher than 50 g/Kg H_2O the increase in inaccuracy resulting from the extrapolation to protein concentration 70 g/Kg H_2O was but small

CORRECTION FOR SYSTEMATIC ERRORS

Two of the errors mentioned when presenting the results of the *in vivo* ultrafiltration experiments may be expected to affect also the results obtained by means of dialysis.

(a) *Flame photometer error in determination of Na and K.* At a protein concentration of about 60 g/Kg H_2O this should lead to underestimating both Na and K concentration in serum by 0.1 mEq per Kg H_2O As a consequence Δ Na and Δ K, when expressed per 100 g protein, are each underestimated by 0 15 mEq, and NCE by 0.3 mEq This error is presumably independent of the pH of the solution analysed.

(b) *Error due to neglect of the presence of carbonate and of carbamino-protein.* Since NCE etc. are calculated from the differences between the concentrations inside and outside the dialysis mats, neglect of the presence of carbonate should cause no error This leaves the error due to neglect of carbamino-protein For reasons given in chapter III we have assumed that at physiological pH and P_{CO_2} sufficient carbamino-protein is present to make us overestimate HCO_3 concentration by 0.3 mEq at a protein concentration of 100 g/Kg H_2O If we accept the existence of this amount of carbamino-protein at pH 7 40, we must expect that below pH 7.00 no carbamino-protein exists, but that at pH 8 00 the concentration will at least be twice that present at pH 7 40 (see experiments of GIUSTINA *et al* 1953)

We conclude that both Δ Na and Δ K are on the average 0.15 mEq too low when expressed per 100 g protein, and that Δ HCO_3 will be correct below pH 7.00, but is overestimated by about 0.3 mEq at pH 7 40 and by about 0 6 mEq at pH 8.00. As a consequence NCE should be underestimated by 0.3 mEq below pH 7 00, by 0 6 mEq at pH 7 40 and by 0.9 mEq at pH 8 00, all values expressed per 100 g protein.

In the following sections only the average values will be corrected for these errors

RESULTS

These may be divided into those concerning NCE and Δ cation and those concerning the concentration ratios Henceforward the terms NCE and Δ cation refer to quantities

expressed per 100 g protein, unless otherwise stated. Similarly the term concentration ratio will indicate the ratio at a protein concentration of 70 g/Kg H_2O

NCE and Δ cation

By expressing all quantities per 100 g protein differences due to variation in actual protein concentration are eliminated and for normal sera the differences in protein composition may be considered negligible. But the effect of pH remains and must be taken into account when comparing the values found for NCE or Δ cation.

NCE

Fig. 49 (left-hand side) graphically presents the values found for NCE in relation to the pH of the serum or plasma (the three experiments in table 47 in which the latter was not determined are not included).

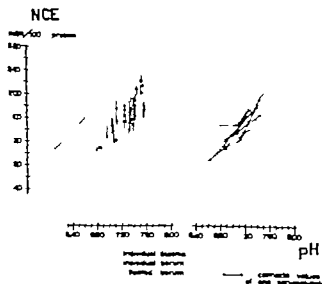


Fig 49 Normal serum and plasma. Correlation between pH and NCE (values not corrected for systematic errors) On the left side all experiments are represented, on the right side only those in which the same serum sample was dialysed at different pH values. The broken line represents the values calculated from the equation given by V SLYKE *et al* in 1928 For further explanation see text.

For each point in the graph the SE was calculated from the SD found for single determination of NCE (table 20 in chapter V) and from the number of units averaged for the calculation of that particular point. For NCE the range covered by ± 2 SE is indicated by the vertical lines projecting upwards and downwards from the symbols. For pH the range ± 2 SE is indicated by the diameter of the symbol. The broken line represents the relationship between NCE and pH according to the 1928 publication of VAN SLYKE *et al* (equation D in chapter II).

In fig. 49 (right-hand side) only the values obtained for sera which were dialysed at more than one pH alue are given. The values found for one serum during one dialysis experiment ar connected.

Th mpression gained from fig. 49 is that the values found for NCE are considerably below the alues expected from Van Slyke s experiments, but that the change per unit pH i about the same.

As long as the concentration of $[Cat]_o$ is large in comparison to Δ Cat (as in the case of Na and K) the relation between concentration ratio and protein concentration is practically linear for protein concentrations up to 100 g/Kg H_2O But if Δ Cat is of the same magnitude as $[Cat]_o$ – as in the case of Ca and Mg – the relation is curvilinear

In chapter I a similar relation was deduced for the Donnan ratio and fig. 48 shows for Na Cl and Ca + Mg the relation experimentally found in the experiments in which various dilutions of the serum were dialysed in one container It can be seen that for Na and Cl the relationship is indeed rectilinear within the error of determination whereas for Ca + Mg the curvilinearity is evident. K and HCO_3 are not shown because of the much larger spread in their case – but the position of the points was found compatible with rectilinearity

From fig 48 the necessity of expressing concentration ratios at uniform protein concentration should be evident and since in the experiments represented in table 47 the actual protein concentration was always higher than 50 g/Kg H_2O the increase in inaccuracy resulting from the extrapolation to protein concentration 70 g/Kg H_2O was but small

CORRECTION FOR SYSTEMATIC ERRORS

Two of the errors mentioned when presenting the results of the *in vivo* ultrafiltration experiments may be expected to affect also the results obtained by means of dialysis.

(a) *Flame photometer error in determination of Na and K* At a protein concentration of about 60 g/Kg H_2O this should lead to underestimating both Na and K concentration in serum by 0.1 mEq per Kg H_2O As a consequence Δ Na and Δ K, when expressed per 100 g protein, are each underestimated by 0.15 mEq, and NCE by 0.3 mEq This error is presumably independent of the pH of the solution analysed.

(b) *Error due to neglect of the presence of carbonate and of carbamino-protein* Since NCE etc. are calculated from the differences between the concentrations inside and outside the dialysis units, neglect of the presence of carbonate should cause no error This leaves the error due to neglect of carbamino-protein. For reasons given in chapter III we have assumed that at physiological pH and P_{CO_2} sufficient carbamino-protein is present to make us overestimate HCO concentration by 0.3 mEq at a protein concentration of 100 g/Kg H_2O If we accept the existence of this amount of carbamino-protein at pH 7 40 we must expect that below pH 7.00 no carbamino-protein exists, but that at pH 8.00 the concentration will at least be twice that present at pH 7 40 (see experiments of GIUSTINA *et al* 1953).

We conclude that both Δ Na and Δ K are on the average 0 15 mEq too low when expressed per 100 g protein, and that Δ HCO_3 will be correct below pH 7 00, but is overestimated by about 0.3 mEq at pH 7 40 nd by about 0.6 mEq at pH 8.00 As a consequence NCE should be underestimated by 0.3 mEq below pH 7 00, by 0 6 mEq at pH 7 40 and by 0.9 mEq at pH 8.00, all alues expressed per 100 g protein.

In the following sections only the average values will be corrected for these errors.

RESULTS

These may be divided into those concerning NCE and Δ cation and those concerning the concentration ratios. Henceforward the terms NCE and Δ cation refer to quantities

Accordingly at pH 7.33 NCE would be calculated at 16.3 mEq/100 g protein The agreement with the corresponding (non-corrected) mean values calculated from the serum samples in the pH range 7 10–7.50 (table 48) is again rather satisfactory

A correction for the effect of the systematic errors can be made in the following manner The error in flame photometry is independent of pH and should therefore not affect the slope of the regression line, but only the intercept with the horizontal axis *i.e* the apparent IEP But the error due to carbamino-protein was assumed to be zero below pH 7.00 and 0.6 mEq/100 g protein at pH 8 00 a correction for this error must therefore increase the slope of the line in the pH range 7.00 to 8 00. Finally although in the experiments used for calculating the average regression line the pH ranged from 6.33 to 7 96, all but three points fall within the range 6.60 to 7 70 Thus we found the best estimate of the relationship between pH and NCE for the pH range 6 60–7 00

$$\text{NCE (mEq/100 g protein)} = 10.0\ (\text{pH} - 5\ 66),$$

for the pH range 7 00–7 70

$$\text{NCE (mEq/100 g protein)} = 10\ 7\ (\text{pH} - 5\ 74).$$

These equations should be valid for serum with normal protein composition and normal electrolyte concentrations

Δ cation

In fig. 50 we have presented the data for Δ Na, Δ K and Δ Ca + Mg in the same manner as for NCE. On the left-hand side all alues (± 2 SE of the mean) for which the pH in serum or plasma was determined are presented, on the right hand side only the results for serum samples dialysed at more than one pH value For Δ K and Δ Ca + Mg some further comment is necessary

In their case the concentration of 'free' ion in the individual experiments differed by as much as 15 % from the mean concentration calculated for all experiments, whereas for Na the difference was always less than 3 Since large changes in the concentration of 'free' ion must affect the correlation between pH and Δ cation, the right-hand side of fig. 50 contains in the case of Δ Ca + Mg only the experiments in which the concentration of Ca + Mg in the dialysis solution was within the range 4 00 to 4 20 mEq/Kg H_2O (mean concentration 4 08). This spread is comparable to that found for the Na concentration of the dialysis solution.

The K concentration in the stock dialysis solution was changed after 14-5-'61 from 3 90 mEq/L to 4 75 mEq/L in attempt to make the influence of the absolute error smaller But for K the percentage error remained considerably larger than for Na and, together with the fact that the flame photometer error is also considerably larger than in the case of Na, this greatly limits the accuracy of the results obtained for K Although fig. 50 (right-hand side) in the case of K only presents the experiments in which the concentration in the dialysis solution was between 4 69 and 5 06 mEq/Kg H_2O no definite pattern emerges and we shall therefore not attempt to correlate Δ K with pH

Table 50 gives the erages of the values obtained for Δ Na, Δ K and Δ Ca + Mg in the same 14 experiments with serum from which the mean NCE value for the pH range 7 10–7 50 was calculated. For each cation the average concentration in the dialysis solution is also gi en Comparing these results with those obtained for protein-bound Na nd Ca + Mg by means of *in ro* ultrafiltration (chapter VI), we find that the values found with dialysis are lower although the concentration of 'free' cation was about the

Average value of NCE at pH of normal venous blood In the *in vivo* ultrafiltration experiments endcompression pH ranged from 7 27 to 7 35 in the normal persons The number of dialysis experiments in which the pH inside the serum or plasma containing unit was within this range is only 5 but if we assume a linear relationship between pH and NCE for the pH range 7 10–7 50 – and this assumption is not unreasonable as fig. 49 shows – 14 observations with serum and 3 with plasma become available for comparison with the results obtained by means of *in vivo* ultrafiltration.

The experiments have been selected as follows. When for one serum sample two experimental points fall within the pH range indicated the value with the pH nearest to 7.30 was chosen. And when serum of the same individual was studied more than once only the result of the first experiment was taken. The pooled sera have all been included, although they contained serum of at least one of the subjects of whom also an individual serum is included in the series. The experiments thus include serum or plasma from 15 different subjects.

In table 48 the experiments are specified according to the solution studied The sera show the lowest average value and plasma the highest. The small number of experiments, the difference in mean pH value and the fact that the samples were not obtained from the same subjects prevent the difference between serum and plasma from being statistically significant It is however in the direction expected from the estimated contribution of heparin to NCE (chapter V) and we have therefore considered the serum values to be the more accurate of the two

Correction for systematic errors brings the mean NCE value of the serum experiments from 16 4 to 17 0 mEq/100 g protein at a mean pH value of 7 33 This value is in close agreement with the average value calculated from the *in vivo* ultrafiltration experiments after correction for systematic errors (chapter VI) In chapter VIII the results of both methods will be compared in more detail

If all observations in which the pH of the protein solution was determined are averaged we find for a mean pH value of 7 17 a mean NCE value of 14 7 (for a total of 45 experiments in which the pH ranged from 6 33 to 7 96) Assuming NCE to increase 10 mEq per 100 g protein per unit pH (see page 39) we calculate a (non-corrected) NCE value of 16 3 at pH 7 33 The difference with the non-corrected value obtained from the 14 experiments in the pH range 7 10–7 50 is negligible and for practical purposes the assumption of a rectilinear relationship between pH and NCE over the pH range studied appears to be justified

Finally it should be mentioned that for the one subject (vLe) from whom serum as well as plasma was studied on several occasions the values found for NCE were equal within the error of determination Obviously the error caused by the addition of heparin will only become evident if a larger number of experiments is compared

Correlation between pH and NCE We have calculated the regression line for NCE on pH for each of the 15 serum samples which were dialysed at more than one pH value They include 4 samples of pooled serum The individual equations are presented in table 49 together with the average equation The slopes of the individual regression lines vary considerably owing to the small number of observations per serum and to the difference in pH range covered But the average equation should be a reasonable approximation of the correlation in our experiments. From table 49 we find

$$\text{NCE (mEq/100 g protein)} = 10\,0\,(\text{pH} - 5\,70)$$

Accordingly at pH 7.33 NCE would be calculated at 16.3 mEq/100 g protein. The agreement with the corresponding (non-corrected) mean values calculated from the serum samples in the pH range 7.10–7.50 (table 48) is again rather satisfactory.

A correction for the effect of the systematic errors can be made in the following manner. The error in flame photometry is independent of pH and should therefore not affect the slope of the regression line, but only the intercept with the horizontal axis *i.e.* the apparent IEP. But the error due to carbamino-protein was assumed to be zero below pH 7.00 and 0.6 mEq/100 g protein at pH 8.00: a correction for this error must therefore increase the slope of the line in the pH range 7.00 to 8.00. Finally, although in the experiments used for calculating the average regression line the pH ranged from 6.33 to 7.96, all but three points fall within the range 6.60 to 7.70. Thus we found the best estimate of the relationship between pH and NCE for the pH range 6.60–7.00

$$\text{NCE (mEq/100 g protein)} = 10.0\ (\text{pH} - 5.66),$$

for the pH range 7.00–7.70

$$\text{NCE (mEq/100 g protein)} = 10.7\ (\text{pH} - 5.74).$$

These equations should be valid for serum with normal protein composition and normal electrolyte concentrations.

Δ cation

In fig. 50 we have presented the data for Δ Na, Δ K and Δ Ca + Mg in the same manner as for NCE. On the left hand side all values (± 2 SE of the mean) for which the pH in serum or plasma was determined are presented; on the right-hand side only the results for serum samples dialysed at more than one pH value. For Δ K and Δ Ca + Mg some further comment is necessary.

In their case the concentration of 'free' ion in the individual experiments differed by as much as 15 from the mean concentration calculated for 8 experiments, whereas for Na the difference was always less than 3%. Since large changes in the concentration of 'free' ion must affect the correlation between pH and Δ cation, the right-hand side of fig. 50 contains in the case of Δ Ca + Mg only the experiments in which the concentration of Ca + Mg in the dialysis solution was within the range 4.00 to 4.20 mEq/Kg H_2O (mean concentration 4.08). This spread is comparable to that found for the Na concentration of the dialysis solution.

The K concentration in the stock dialysis solution was changed after 14-5-'61 from 3.50 mEq/L to 4.75 mEq/L in an attempt to make the influence of the 'absolute' error smaller. But for K the percentage error remained considerably larger than for Na and, together with the fact that the flame photometer error is also considerably larger than in the case of Na, this greatly limits the accuracy of the results obtained for K. Although fig. 50 (right-hand side) in the case of K only presents the experiments in which the concentration in the dialysis solution was between 4.69 and 5.06 mEq/Kg H_2O no definite pattern emerges and we shall therefore not attempt to correlate Δ K with pH.

Table 50 gives the averages of the values obtained for Δ Na, Δ K and Δ Ca + Mg in the same 14 experiments with serum from which the mean NCE value for the pH range 7.10–7.50 was calculated. For each cation the average concentration in the dialysis solution is also given. Comparing these results with those obtained for protein-bound Na and Ca + Mg by means of *in vivo* ultrafiltration (chapter VI), we find that the values found with dialysis are lower although the concentration of 'free' cation was about the

Average value of NCE at pH of normal venous blood In the *in vivo* ultrafiltration experiments endcompression pH ranged from 7.27 to 7.35 in the normal persons The number of dialysis experiments in which the pH inside the serum or *plasma containing unit* was within this range is only 5 but if we assume a linear relationship between pH and NCE for the pH range 7 10–7.50 – and this assumption is not unreasonable as fig. 49 shows – 14 observations with serum and 3 with plasma become available for comparison with the results obtained by means of *in vivo* ultrafiltration

The experiments have been selected as follows. When for one serum sample two experimental points fall within the pH range indicated the value with the pH nearest to 7.30 was chosen. And when serum of the same individual was studied more than once only the result of the first experiment was taken. The pooled sera have all been included, although they contained serum of at least one of the subjects of whom also an individual serum is included in the series. The experiments thus include serum or plasma from 15 different subjects.

In table 48 the experiments are specified according to the solution studied. The sera show the lowest average value and plasma the highest. The small number of experiments, the difference in mean pH value and the fact that the samples were not obtained from the same subjects prevent the difference between serum and plasma from being statistically significant. It is, however in the direction expected from the estimated contribution of heparin to NCE (chapter V) and we have therefore considered the serum values to be the more accurate of the two

Correction for systematic errors brings the mean NCE value of the serum experiments from 16 4 to 17 0 mEq/100 g protein at a mean pH value of 7.33 This value is in close agreement with the average value calculated from the *in vivo* ultrafiltration experiments after correction for systematic errors (chapter VI) In chapter VIII the results of both methods will be compared in more detail.

If all observations in which the pH of the protein solution was determined are averaged we find for a mean pH value of 7 17 a mean NCE value of 14 7 (for a total of 45 experiments in which the pH ranged from 6 33 to 7 96) Assuming NCE to increase 10 mEq per 100 g protein per unit pH (see page 39) we calculate a (non-corrected) NCE value of 16 3 at pH 7 33 The difference with the non-corrected value obtained from the 14 experiments in the pH range 7 10–7 50 is negligible and for practical purposes the assumption of a rectilinear relationship between pH and NCE over the pH range studied appears to be justified

Finally it should be mentioned that for the one subject (vLe) from whom serum as well as plasma was studied on several occasions the values found for NCE were equal within the error of determination Obviously the error caused by the addition of heparin will only become evident if a larger number of experiments is compared.

Correlation between pH and NCE. We have calculated the regression line for NCE on pH for each of the 15 serum samples which were dialysed at more than one pH value They include 4 samples of pooled serum The individual equations are presented in table 49 together with the average equation The slopes of the individual regression lines vary considerably owing to the small number of observations per serum and to the difference in pH range covered But the average equation should be a reasonable approximation of the correlation in our experiments From table 49 we find

$$\text{NCE (mEq/100 g protein)} = 10\ 0\ (\text{pH} - 5\ 70)$$

Accordingly at pH 7.33 NCE would be calculated at 16.3 mEq/100 g protein. The agreement with the corresponding (non-corrected) mean values calculated from the serum samples in the pH range 7 10–7.50 (table 48) is again rather satisfactory

A correction for the effect of the systematic errors can be made in the following manner The error in flame photometry is independent of pH and should therefore not affect the slope of the regression line, but only the intercept with the horizontal axis *i.e* the apparent IEP But the error due to carbamino-protein was assumed to be zero below pH 7 00 and 0.6 mEq/100 g protein at pH 8.00 a correction for this error must therefore increase the slope of the line in the pH range 7 00 to 8 00 Finally although in the experiments used for calculating the average regression line the pH ranged from 6.33 to 7 96, all but three points fall within the range 6.60 to 7 70. Thus we found the best estimate of the relationship between pH and NCE for the pH range 6 60–7 00

$$\text{NCE (mEq/100 g protein)} = 10.0\ (\text{pH} - 5\ 66),$$

for the pH range 7 00–7 70

$$\text{NCE (mEq/100 g protein)} = 10.7\ (\text{pH} - 5\ 74).$$

These equations should be valid for serum with normal protein composition and normal electrolyte concentrations.

Δ cation

In fig. 50 we have presented the data for Δ Na, Δ K and Δ Ca + Mg in the same manner as for NCE. On the left-hand side all values (± 2 SE of the mean) for which the pH in serum or plasma was determined are presented, on the right-hand side only the results for serum samples dialysed at more than one pH value For Δ K and Δ Ca + Mg some further comment is necessary

I their case the concentration of 'free' ion in the individual experiments differed by as much as 15 % from the mean concentration calculated for all experiments, whereas for Na the difference was always less than 3 %. Since large changes in the concentration of 'free ion must affect the correlation between pH and Δ cation, the right-hand side of fig. 50 contains in the case of Δ Ca + Mg only the experiments in which the concentration of Ca + Mg in the dialysis solution was within the range 4 00 to 4.20 mEq/Kg H_2O (mean concentration 4 08) This spread is comparable to that found for the Na concentration of the dialysis solution.

The K concentration in the stock dialysis solution was changed after 14-5-'61 from 3.50 mEq/L to 4 5 mEq/L in an attempt to make the influence of the absolute error smaller But for K the percentage error remained considerably larger than for Na and, together with the fact that the flame photometer error is also considerably larger than in the case of Na, this greatly limits the accuracy of the results obtained for K. Although fig. 50 (right-hand side) in the case of K only presents the experiments in hich the concentration in the dialysis solution was between 4 69 and 5 06 mEq/Kg H_2O no definite pattern emerges and shall therefore not attempt to correlate Δ K ith pH

Table 50 gi es the averages of the values obtained for Δ Na, Δ K and Δ Ca + Mg in the same 14 experiment with serum from which the mean NCE alue for the pH range 7 10–7.50 was calculated For each cation the average concentration in the dialysis solution is also given. Comparing these results with those obtained for protein-bound Na and Ca + Mg by means of m l ultrafiltration (chapter VI), we find that the values found with dialysis are lower although the concentration of 'free cation was about the

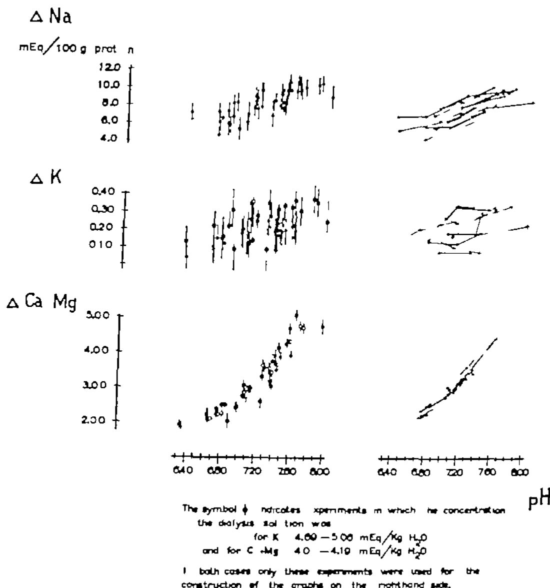


Fig. 50 – Normal serum and plasma. Correlation between pH and respectively ΔNa, ΔCa + Mg (values not *corrected for systematic errors). See subscript fig. 49 and text for explanation.*

same A further discussion will follow in chapter VIII but it can already be said here *that the discrepancy must be due to the effect of the Donnan equilibrium.*

Correlation between pH and Δ cation Fig. 50 demonstrates that whereas for Δ Na the correlation is roughly rectilinear it is clearly curvilinear for Δ Ca + Mg

For Δ Na we have calculated the following average regression line from the same 15 experiments as for NCE

$$\Delta \text{ Na (mEq/100 g protein)} = 4\ 00\ (\text{pH} - 5\ 08)$$

For the individual regression lines the correlation coefficient ranged from 2.34 to 5 54 If we correct for the error in flame photometry the apparent IEP becomes 5 10

For Δ Ca + Mg we have calculated the line of best fit in the following manner First

the data from experiments in which the 'free' Ca + Mg concentration was between 4.00 and 4.26 mEq/Kg H_2O were grouped according to pH, and for each range a mean value was calculated (only experiments with serum were included). Then the same was done for all experiments. Table 51 shows that the mean concentration of Ca + Mg in the dialysis solution was the same for both groups of experiments and that practically the same mean values were found both for Δ Ca + Mg and for pH in all pH ranges (fig. 51). Whereas between pH 6.60 and 6.80 Δ Ca + Mg increases by about 0.1 mEq

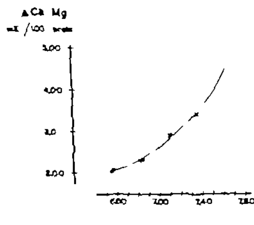


Fig. 51. Normal serum. Correlation between mean values of pH and of ΔCa + Mg for different pH ranges. The crosses refer to the values given in table 51, column I, and the open circles to those given in table 51, column II.

per 0.1 pH unit, the increase is 0.2 mEq over the pH range 7.00–7.20 and something like 0.4 mEq over the range 7.40–7.60. However, within the pH range 7.00–7.50 the relationship can be described approximately by the equation

$$\Delta \text{ Ca} + \text{Mg (mEq/100 g protein)} = 2.50\,(\text{pH} - 5.94).$$

Finally it must be stressed that in order to obtain the correlation between protein-bound cation and pH, the observed correlation between Δ cation and pH has yet to be corrected for the effect of the Donnan equilibrium.

Concentration ratios

Table 47 gives both the ratios obtained with serum or plasma and those obtained with distilled water. The latter should be 1.000 within the error of the determination and thus serve as a 'blank' for the ratios found in the case of serum. Fig. 52 shows for each of the ions studied the relationship between concentration ratio and pH.

All experiments have been included with the exception of those in which the pH of the serum or plasma was not determined. The points refer to 'distilled water', the crosses to serum or plasma. Crosses representing the same serum sample are connected.

It is obvious that the values obtained for R_K and for R_{HCO_3} are subject to a large accidental error, but for R_{Na}, R_{Cl} and R_{Ca} the spread is relatively small in comparison to the influence of pH. In the case of these ions even the individual serum samples show roughly linear correlation between concentration ratio and pH. We have therefore

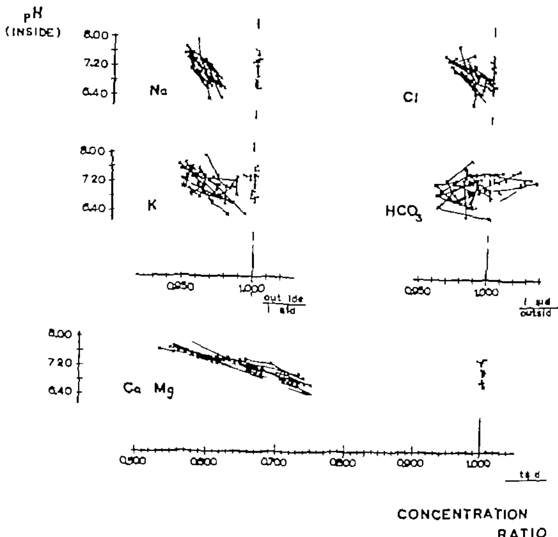


Fig. 52 Normal serum and plasma. Correlation between pH and the concentration ratios for the equilibrium dialysis solution serum (crosses) and for the equilibrium dialysis solution distilled ater' (points). V lues not corrected for systematic errors.

divided our observations on *serum* in two groups according to pH value For the pH ranges 6 60–7 20 respectively 7 20–7 75 mean values were calculated for each of the concentration ratios and for the pH by simply averaging the values within one group The results are presented in table 52 Since these data will later be used for the calculation of the amount of ion that is bound in a complex-type manner (chapter VIII), we have estimated the SE of the mean for the concentration ratio of each ion

For the equilibrium dialysis solution-serum we proceeded as follows. On the assumption that within the pH range 6.60 to 7 75 a rectilinear correlation exists between pH and the mean alue of the concentration ratio of the different ions we have calculated for R , R_K etc. the mea lue to be expected at the pH value of each individual experiment from the mean alues given in table 52 for pH 6.91 and 7 46 respectively We then calculated for every individual value of R_{Na}, R etc the deviation from the mean at the particula pH alue Knowing these we could calculate for each of the concentration ratios the SD of a individual alue and the SE of the mean for the pH ranges 6.60–7.20 and 7.20–7 75 This manner of calculating the spread is rather crude and in the case of R_{Ca}, M the assumption of a rectilinear correlation with pH is only correct for the

smaller pH range 7.00 to 7.50. Nevertheless the values estimated for the SE of the mean should be reasonably accurate, since we found nearly the same values when we calculated for R, R etc the SE of the mean to be expected from accidental errors (for which calculation we used the data given in tables 28, 30 and 31 in chapter V)

The ratios for the equilibrium dialysis solution 'distilled water' are not affected by pH and in this case the SE of the mean could be calculated for R, R etc in the usual manner

Table 52 confirms the impression gained from fig. 52. For the equilibrium dialysis solution-distilled water the mean values of the ratios are well within probable range of 1 000 For the equilibrium dialysis solution-serum we find in the case of R_{Na}, R_{Cl} and R_{Ca+Mg} an apparently significant difference between the mean values calculated for the two different pH ranges, but in the case of R_{HCO_3} the spread is very large. We can only conclude that on the average R_{HCO_3} appears to be of the same order as R_{Cl}. For R_K the situation is complicated by the fact that part of the experiments were performed at a 'free' K concentration of about 3 50 mEq/Kg H_2O the remainder at a concentration of about 4 75 mEq/Kg H_2O But this difficulty is a minor one compared with the problem of the systematic errors.

Table 53 shows the effect of correcting the mean values of R_{Na} and of R_K for the *flame photometer error In the case of R_K the magnitude of the correction is less than* 0 1 %, but for R_K it amounts to about 2 of the mean value. The fact that the magnitude of the flame photometer error is less well substantiated for K than for Na further increases the inaccuracy of the values obtained in the case of K As to the systematic error due to neglect of the presence of carbamino-protein, it must be stated again that the magnitude of the correction is very much an estimate. But the general effect of such a correction would be to decrease the mean concentration ratio of HCO_3 at pH 7 44 to about 0 975 and to leave the concentration ratio at 6 91 unaffected.

On the assumption that for R_{Na}, R_{Cl} and R_{Ca+Mg} a rectilinear relationship exists with pH over the pH range 6 90 to 7 40 we could make an estimate of the correlation with the aid of table 52. Thus we found

$$R_{Na} = 1\,000 - 0.018\,(pH - 4\,98)$$
$$R_{Ca+Mg} = 1.000 - 0\,185\,(pH - 5.30)$$
$$R_{Cl} = 1\,000 - 0\,029\,(pH - 6.60)$$

The most interesting finding is that whereas R and R_{Ca+Mg} become 1 000 at a pH value near 5 00, the corresponding pH value is 6.60 in the case of R_{Cl} But a detailed discussion of the implications of the results obtained for the concentration ratios must wait till chapter VIII

Data concerning Δ Ca and Δ Ca + Mg, and R_{Ca} and R_{Mg}

In four experiments with plasma both Ca and Ca + Mg were determined The results are summarised in table 54 When the effect of pH is taken into account both for Ca and for Mg about the same percentage binding is found as calculated from the *in vivo* ultrafiltration experiments (table 43 in chapter VI). Since relatively more Ca than Mg is bound, R_{Ca} is somewhat lower than R_{Mg}.

SERUM FROM PATIENTS

Only four patients were studied but the results obtained merit some attention

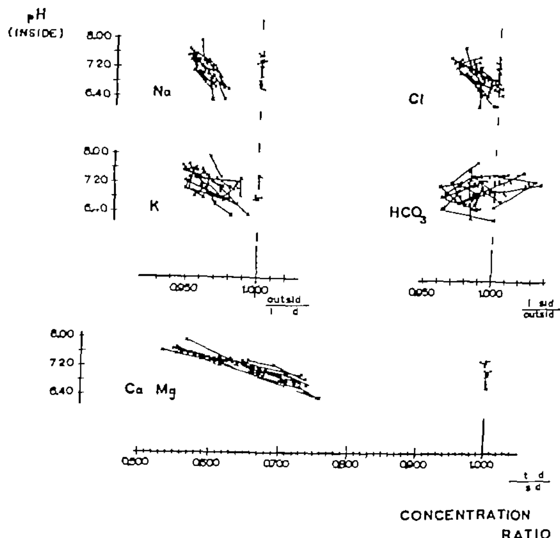


Fig. 52 Normal serum and plasma. Correlation between pH and the concentration ratios for the equilibrium dialysis solution – serum (crosses) and for the equilibrium dialysis solution distilled water' (points). Values not corrected for systematic errors.

divided our observations on *serum* in two groups according to pH value For the pH ranges 6 60–7.20 respectively 7 20–7 75 mean values were calculated for each of the concentration ratios and for the pH by simply averaging the values within one group The results are presented in table 52. Since these data will later be used for the calculation of the amount of ion that is bound in a complex type manner (chapter VIII), we have estimated the SE of the mean for the concentration ratio of each ion

For the equilibrium dialysis solution-serum we proceeded as follows. On the assumption that within the pH range 6.60 to 7 75 a rectilinear correlation exists between pH and the mean value of the concentration ratio of the different ions we have calculated for R , R_K etc. the mean value to be expected at the pH value of each individual experiment from the mean values given in table 52 for pH 6.91 and 7.46 respectively We then calculated for every individual value of R , R etc the deviation from the mean at the particular pH value knowing these we could calculate for each of the concentration ratios the SD of an individual value and the SE of the mean for the pH ranges 6 60–7.20 and 7.20–7 75 This manner of calculating the spread is rather crude and in the case of R_{Ca} the assumption of a rectilinear correlation with pH is only correct for the

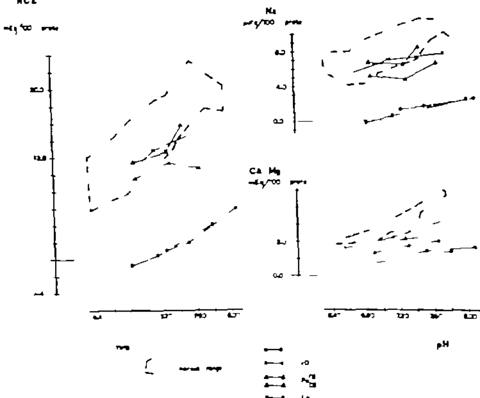


Fig. 53 Serum from patients. Correlation between pH and respectively NCE, Δ Na, Δ Ca + Mg (values not corrected for systematic errors). The area indicated as normal range includes ±2 SE for the individual points shown in figs. 49 and 50.

PURIFIED PROTEIN FRACTIONS

DATA CONCERNING PREPARATIONS

The first experiments were done with bovine albumin and one sample of human placentar γ-globulin, but towards the end of our investigations we have also studied albumin and γ-globulin prepared from human serum. The following preparations were used

Albumin

Bovine plasma albumin (Povitel). The fractions are prepared from citrate plasma. After precipitation of globulins (ethanol 40 %, pH 5.8), the pH of the supernatant is changed to 5.2. The precipitate which then forms contains albumin, α- and β-globulins. After dissolving the precipitate in water α- and β-globulins are precipitated (10 % ethanol, pH 4.5–4.7) and from the supernatant albumin is finally precipitated (40 % ethanol, pH 4.5). The paste is redissolved in water, stabilised and pasteurised. During this stage of the procedure a new precipitate often forms (probably α-globulins), which is removed. The resulting solution is freed from electrolytes by passing it over an anion-exchanging resin, then over a cation-exchanging resin, after which the pH of the solution

Clinical data In two patients (Em, La) the diagnosis multiple myeloma was proven beyond doubt (histology of bone marrow roentgenologic picture of bone lesions, paper electrophoresis of serum) and in both the paraprotein was a γ-globulin. In the case of patient La this was confirmed by immune electrophoresis. The third patient (Po) – courtesy of Dr J Gerbrandy and Dr S. T Boen – presented with a fairly typical clinical picture and a large amount of polymorphic plasma cells in the bone marrow but no bone lesions were seen roentgenologically The serum contained 130 g/L protein, 80 / of which was present as a typical γ-myeloma band on the electrophoresis strip With the Majoor technique the abnormal protein was found in the euglobulin fraction. Finally in the fourth patient (vO) – courtesy of Dr N Lubsen – the clinical picture and the laboratory findings, including needle biopsy from a solitary swollen lymph gland, suggested carcinomatosis, primary tumor unknown. In this case a pathological increase in $β_2M$-globulin was found with the aid of immuno-electrophoresis (Dr F Peetoom) the other immuno-globulins showing a decrease in concentration. On the strength of this finding the abnormality was classified as a macro-globulinaemia. With the Majoor technique the pathological protein was found to behave as a pseudo-globulin, whereas with paper electrophoresis the fraction was found at the place of the $β_2$-globulins. This combination of findings obtained with immuno-electrophoresis, paper electrophoresis and the method of Majoor remained unaltered during the further course of the disease. Dr F Peetoom commented upon the peculiar behaviour of the pathological protein since in his experience $β_2M$-globulin, contrary to $β_2A$-globulin, never behaves as a pseudo-globulin.

RESULTS

These are listed at the end of table 47 in the same way as the results obtained for normal subjects

NCE and Δ cation

From fig. 53 it is evident that in as far as NCE and Δ Na are concerned, the four patients studied may be divided into two groups the two patients with an unequivocal γ myeloma having very low and at pH 6 80 even zero values, the patient with macroglobulinaemia and the patient with probable γ myeloma having normal to low normal values. For Δ Ca + Mg the differences are less pronounced. For Δ K (not reproduced in fig. 53) the findings are on the whole comparable with those for Δ Na.

Concentration ratios

In the case of the two patients with typical γ myeloma we found that whereas Rx becomes 1 000 at about pH 7 00 R_{Cl} becomes higher than 1 000 – in all probability because NCE tends to become negative below that pH and the net charge of the serum proteins therefore positive A rather interesting finding is the fact that R_{Ca+Mg} remains considerably below 1 000 even at pH values of about 7 00 For the sera from the two other patients the differences in regard to normal sera are only slight.

The small number of sera notwithstanding, two points emerge from these experiments.

Apparently patients who clinically and according to routine paper electrophoresis are classified as cases of γ myeloma can differ considerably in the cation binding properties of their proteins (Po as against patients Em and La) But in general the dialysis experiments confirm the *in vivo* ultrafiltration finding that the paraproteins of a γ myeloma have a low value for NCE.

A significant amount of Ca + Mg is still bound by serum protein at a pH where both the behaviour of the univalent cations and the value found for NCE indicates that protein net charge has become zero or even positive

The results of the individual experiments are presented in table 55 which was constructed in the same manner as table 47

In the first two series of experiments in which human albumin and γ-globulin from the Red Cross Laboratory were dialysed the dialysis solution contained about 1-2 mEq/L citrate, and both in the dialysis solution and in the protein solution 1 to 2 mEq/L Ca + Mg must therefore have been present as a citrate-complex. Although the decrease in ionised Ca + Mg should also influence the values obtained for NCE in the sense that the latter become somewhat smaller it is unlikely that this error was more than 0.5 mEq/100 g protein (compare Δ Ca + Mg for the experiments with and without citrate).

In table 55 one series of experiments is listed in which Na and K were determined after deproteinisation (6 / trichloroacetic acid 1 21). The experiment is included because of the results obtained for Δ Ca + Mg.

Finally the experiment listed at the bottom of table 55 represents a special case and will be discussed in one of the following sections.

Experiments concerning interaction between albumin and γ-globulin in the physiological pH range – Its possible effect on the determination of NCE and Δ cation

In chapter I and in the discussion of the *in vivo* ultrafiltration method we already mentioned the possibility that protein-protein interaction might occur during haemoconcentration and render difficult the interpretation of the results obtained. Since the IEP of the various plasma proteins differs considerably it is conceivable that part of the negative charges on albumin are neutralised by positive charges on the γ-globulin molecules This in itself should not affect our calculation of the NCE of plasma proteins considered as a whole, provided the degree of protein-protein interaction remains proportionally the same during haemoconcentration. Since (in a solution containing various protein fractions) we can only determine the net ion equivalency for all proteins together the value found for NCE will be the same whether part of the charge on protein molecules is neutralised by oppositely charged sites on other protein molecules or whether both are neutralised by filtrable cations and anions. However this is only true if the charged sites on the molecules act in regard to each other in the same manner as in regard to the filtrable ions If for instance, loose aggregates would form with concomitant change in the structural arrangement of ionised and non-ionised groups on the protein surface, the net charge of the aggregate might conceivably differ from the sum of the net charges of each of its components. In that case any extrapolation from values obtained at one total protein concentration to those to be expected at another total protein concentration is bound to be erroneous and so would the prediction of NCE of a protein mixture from the values obtained for the component parts

The latter possibility is open to investigation We have proceeded in the following manner Solutions of albumin, of γ-globulin and of a mixture of both were dialysed in one container The various protein solutions were therefore exposed to identical conditions In table 56 the values calculated for the mixtures of albumin and γ-globulin (from their relative concentrations and from the values *found* for each protein fraction separately) are compared with the values actually found.

is between 4.5–4.8 and the conductivity between 50.000–90 000 Ω. The solution is finally freeze dried (data kindly supplied by Dr G G A. Mastenbroek)

We received the albumin in a 10 / aqueous solution freshly prepared from the freezedried stock substance and sterilised by passage through Seitz filters. Solutions from two batches were studied. The first (batch 110) was received 10-5- 60, the second (batch 123) 5-5- 61 Between experiments the solutions were stored at 6°C and no bacterial contamination could be detected even after one year storage. With paper electrophoresis the solution was found to contain albumin with only traces of the globulins.

Human plasma albumin (Central Laboratory of the Netherlands Red Cross Bloodtransfusion Service). At our request Dr H W Krijnen was kind enough to send us two albumin preparations.

(1) *Standard albumin preparation, which is obtained from fraction V (cold-ethanol method) 6* by first precipitating α and β-globulins in an ethanol-ether mixture at pH 4.5 and then precipitating albumin from the supernatant by 40 / ethanol pH 4.5

(2) *Albumin extra purified* by redissolving the standard albumin preparation and then treating it for a second time with ethanol-ether at pH 4.5

Both preparations were dissolved in water pasteurised and sent to us without delay They ere received 6-3- 62, kept at —15°C for 2 weeks, then thawed at 6°C and kept at that temperature during 3 to 4 days before use The pH of the final solution was 7 40 to 7.50, the citrate content 9–12 mM/L. With paper electrophoresis both preparations were found to contain albumin with only traces of globulin.

As a result of a misunderstanding we were not prepared for the high citrate content of the solutions, with the result that the final citrate content in the containers was in the first experiments of the order of 0.5 mM/L. For that reason we also prepared

(3) *Solution of standard albumin preparation containing less than 2 mM/L citrate* The solution of standard albumin preparation described above was dialysed against distilled water during 24 hours at 7°C. At the end of this period the protein solution contained less than 2 mM/L citrate and in the dialysis experiment subsequently performed with this albumin solution the final citrate content in the containers must have been less than 0.1 mM/L.

Human albumin crystallised (Nutritional Biochemical Corporation). A commercial sample was used It is a flaky white powder which was kept sealed and stored at 5 C until immediately before the dialysis experiment – 6 g of the powder was dissolved in 60 ml of sterile stock dialysis solution at pH 7 60, resulting in a clear fluid with a brownish tinge. With paper electrophoresis the solution was found to contain albumin and surprisingly a small amount of β-globulin (about 5 / of total protein).

Gamma-globulin

Human placentar γ-globulin (Poviet) By courtesy of Dr G G A. Mastenbroek we received a sample of dried placentar γ-globulin, prepared by a modification of cold ethanol methods 6 and 9 A 10 stock solution was prepared by dissolving the powder in 0 9 NaCl solution. The stock solution was prepared in May 1960 and kept stored at 5°C in a sterile container W th paper electrophoresis only γ-globulin was found

Human plasma γ-globulin (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service) The standard γ-globulin preparation was used We received it 6-3- 62 as a 7 aqueous solution containing 1 mM citrate/L. It was stored and treated as described for the albumin preparations from the same laboratory With paper electrophoresis no other protein fraction could be detected.

Human serum γ-globulin (Serva). A commercial sample was used, marketed as γ-globul n lyoph. pure salt free (fract II) It is fine white powder which we kept sealed and stored at 5 C Immediately before use 5 g was dissolved in 50 ml of sterile stock dialysis solution at pH 7 60, resulting in a whitish opalescent fl id. With paper electrophoresis only γ-globulin was found.

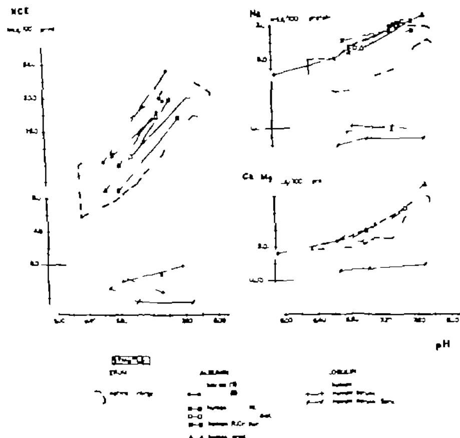


Fig 54 Albumin and γ-globulin Correlation between pH and respectively NCE, Δ Na, Δ Ca + Mg (values not corrected for systematic errors) For area indicated as normal range see subscript fig. 53.

protein content. A somewhat similar conclusion was drawn in chapter VI from the *in vitro* ultrafiltration results obtained in patients with an abnormal composition of serum proteins.

In an attempt to verify the above conclusion concerning the NCE for the protein fractions other than albumin and γ-globulin the following pilot experiment was done with the aid of Dr Krijnen of the Central Laboratory of the Netherlands Red Cross Bloodtransfusion Service.

In his laboratory one batch of human plasma was divided in 4 equal portions. One portion was immediately lyophilized. The other three portions were subjected to the ethanol-cold fractionation method in use at the laboratory in the following manner On the first of these portions only the first step of the fractionation was carried out, resulting in a precipitate containing fraction I and a supernatant containing all other fractions (II III–IV V). The supernatant was lyophilized. For the second of the three portions the fractionation was carried one step further resulting in a supernatant containing fraction IV and V Again the supernatant was lyophilized. The third portion of plasma was carried through three steps and the supernatant, containing fraction V lyophilized. It was thought that by using the supernatant only damage to the various fractions which presumably occurs most easily during precipitation could be avoided.

The relative proportion of the two fractions in the mixture was calculated in the following manner From table 55 it can be seen that in nearly all experiments presented in table 56 albumin and γ-globulin were sufficiently pure to accept the alb/(alb+euglob) ratio respectively alb/(alb+γ-glob) ratio found in the mixed solutions as indicative of the true relative proportions. The fact that with the method of Majoor the mixed solutions appear to contain a much larger amount of pseudo-globulin than is found in the pure fractions, is no objection to this procedure, as in the experiments of 23-3- 62 and 26-3- 62 practically identical relative proportions were calculated for albumin from the results obtained with electrophoresis and from those obtained by the method of Majoor This indicates that albumin and euglobulin contribute to pseudo-globulin in relation to their relative concentrations. (Incidentally this sudden creation of pseudo-globulin by mixing albumin and γ-globulin stresses the need for testing the effect of this mixing on NCE and Δ cation calculated for total protein)

However in the series of experiments performed on 25-10- 62 the albumin used contained traces of α-globulin, but a fairly large amount of β-globulin, whereas the γ-globulin was comparable in purity to the samples used in earlier experiments. In this case therefore the percentage albumin given in table 56 indicates in fact (alb + α + β-globulin) as a percentage of (alb + α- + β- + γ-globulin)

For NCE and Δ Na the differences between calculated and observed values are of the order of the error of determination For Δ Ca + Mg the values observed are on the whole somewhat higher than those calculated but in view of the cumulative errors involved the difference is of doubtful significance We conclude that under the conditions of our experiments and in as far as the determination of NCE and protein-bound cation is concerned the protein fractions act as independent macromolecules.

RESULTS

NCE and Δ cation

The difference between the values obtained for albumin and for γ globulin is evident from fig 54 The latter contains for NCE and Δ Na all values given in table 55 but for Δ Ca + Mg only those from the experiments in which no or practically no citrate was present in the dialysis solution

According to the results obtained for NCE and Δ Na, γ-globulin is positively charged at physiological pH, with a net anion equivalency of about 1–2 mEq/100 g protein (in the graph expressed as a negative value for NCE) In accordance with this the pH was generally higher in the γ globulin solution than in the dialysis solution. At the same time however in all experiments performed with γ globulin Ca and Mg were found to be bound in no uncertain manner

For the albumin fractions the most important finding is that for all preparations studied the value of NCE was within the same range as the values found for normal serum whereas Δ Na and Δ Ca + Mg were on the whole slightly higher although the Na and Ca + Mg concentrations in the dialysis solution were the same in the experiments with protein fractions and in those with serum Two conclusions seem inescapable. The first one is that per unit weight, albumin binds more Cl and/or HCO_3 than the mixture of protein fractions present in serum We will return to this when discussing the concentration ratios. The second conclusion is that one or more fractions of α_1 α_2- and β-globulins must have an equal or higher NCE per unit weight than albumin Otherwise serum could not have the same NCE per 100 g of protein as albumin, since γ-globulins – which apparently have a negative value for NCE – make up 15 % of serum

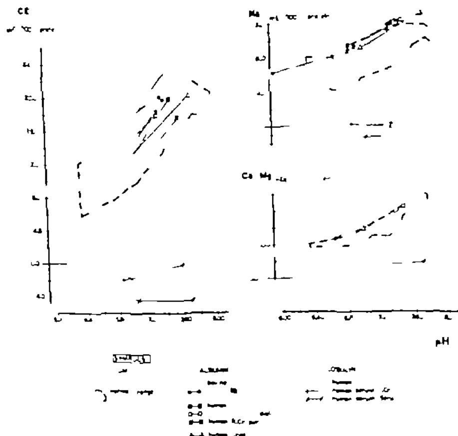


Fig 54 Albumin and γ-globulin Correlation between pH and respectively NCE, Δ Na, Δ Ca + Mg (values not corrected for systematic errors) For area indicated as normal range see subscript fig 53

protein content. A somewhat similar conclusion was drawn in chapter VI from the *in vivo* ultrafiltration results obtained in patients with an abnormal composition of serum proteins

In an attempt to verify the above conclusion concerning the NCE for the protein fractions other than albumin and γ-globulin the following pilot experiment was done with the aid of Dr Krijnen of the Central Laboratory of the Netherlands Red Cross Bloodtransfusion Service.

In his laboratory one batch of human plasma was divided in 4 equal portions. One portion was immediately lyophilized. The other three portions were subjected to the ethanol-cold fractionation method in use at the laboratory in the following manner On the first of these portions only the first step of the fractionation was carried out, resulting in a precipitate containing fraction I and a supernatant containing all other fractions (II–III IV V). The supernatant was lyophilized. For the second of the three portions the fractionation was carried one step further resulting in a supernatant containing fraction IV and V Again the supernatant was lyophilized. The third portion of plasma was carried through three steps and the supernatant, containing fraction V lyophilized. It was thought that by using the supernatant only damage to the various fractions which presumably occurs most easily during precipitation could be avoided.

The relative proportion of the two fractions in the mixture was calculated in the following manner From table 55 it can be seen that in nearly all experiments presented in table 56 albumin and γ-globulin were sufficiently pure to accept the alb/(alb + euglob) ratio, respectively alb/(alb + γ-glob) ratio found in the mixed solutions as indicative of the true relative proportions. The fact that with the method of Majoor the mixed solutions appear to contain a much larger amount of pseudo-globulin than is found in the pure fractions, is no objection to this procedure, as in the experiments of 23-3- 62 and 26-3- 62 practically identical relative proportions were calculated for albumin from the results obtained with electrophoresis and from those obtained by the method of Majoor This indicates that albumin and euglobulin contribute to pseudo-globulin in relation to their relative concentrations. (Incidentally this sudden creation of pseudo-globulin by mixing albumin and γ-globulin stresses the need for testing the effect of this mixing on NCE and Δ cation calculated for total protein)

However in the series of experiments performed on 25-10- 62 the albumin used contained traces of -globulin, but a fairly large amount of β-globulin, whereas the γ-globulin was comparable in purity to the samples used in earlier experiments. In this case therefore the percentage albumin given in table 56 indicates in fact (alb + α- + β-globulin) as a percentage of (alb + α- + β- + γ-globulin).

For NCE and Δ Na the differences between calculated and observed values are of the order of the error of determination. For Δ Ca + Mg the values observed are on the whole somewhat higher than those calculated but in view of the cumulative errors involved the difference is of doubtful significance We conclude that under the conditions of our experiments and in as far as the determination of NCE and protein bound cation is concerned the protein fractions act as independent macromolecules.

RESULTS

NCE and Δ cation

The difference between the values obtained for albumin and for γ-globulin is evident from fig. 54 The latter contains for NCE and Δ Na all values given in table 55 but for Δ Ca + Mg only those from the experiments in which no or practically no citrate was present in the dialysis solution

According to the results obtained for NCE and Δ Na, γ globulin is positively charged at physiological pH with a net anion equivalency of about 1–2 mEq/100 g protein (in the graph expressed as a negative value for NCE) In accordance with this the pH was generally higher in the γ globulin solution than in the dialysis solution At the same time however in all experiments performed with γ-globulin Ca and Mg were found to be bound in no uncertain manner

For the albumin fractions the most important finding is that for all preparations studied the value of NCE was within the same range as the values found for normal serum, whereas Δ Na and Δ Ca + Mg were on the whole slightly higher although the Na and Ca + Mg concentrations in the dialysis solution were the same in the experiments with protein fractions and in those with serum Two conclusions seem inescapable The first one is that, per unit weight, albumin binds more Cl and/or HCO_3 than the mixture of protein fractions present in serum We will return to this when discussing the concentration ratios. The second conclusion is that one or more fractions of α_1 α_2- and β-globulins must have an equal or higher NCE per unit weight than albumin Otherwise serum could not have the same NCE per 100 g of protein as albumin since γ globulins – which apparently have a negative value for NCE – make up 15 % of serum

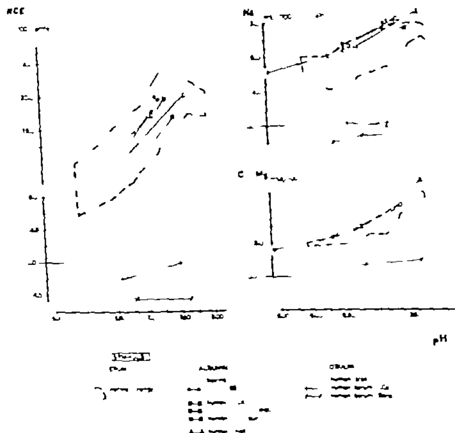


Fig 54 Albumin and γ-globulin Correlation between pH and respectively NCE, Δ Na, Δ Ca + Mg (values not corrected for systematic errors) For area indicated as normal range see subscript fig 53

protein content. A somewhat similar conclusion was drawn in chapter VI from the *in vivo* ultrafiltration results obtained in patients with an abnormal composition of serum proteins.

In an attempt to verify the above conclusion concerning the NCE for the protein fractions other than albumin and γ-globulin the following pilot experiment was done with the aid of Dr Krijnen of the Central Laboratory of the Netherlands Red Cross Bloodtransfusion Service.

In his laboratory one batch of human plasma was divided in 4 equal portions. One portion was immediately lyophilized The other three portions were subjected to the ethanol-cold fractionation method in use at the laboratory in the following manner On the first of these portions only the first step of the fractionation was carried out, resulting in a precipitate containing fraction I and supernatant containing all other fractions (II–III IV V) The supernatant was lyophilized. For the second of the three portions the fractionation was carried one step further resulting in supernatant containing fraction IV and V Again the supernatant was lyophilized. The third portion of plasma was carried through three steps and the supernatant, containing fraction V lyophilized It was thought that by using the supernatant only damage to the various fractions which presumably occurs most easily during precipitation could be avoided.

The 3 sterile flasks, containing the supernatant of successive steps in the fractionation procedure in a lyophilised state, were stored at —15°C for 4 weeks. Then their contents were redissolved in an amount of distilled water calculated to bring the final protein concentration in each flask to ± 70 g/L. Following this the contents of each flask were dialysed against distilled water for 36 hours (at 30°C and under sterile conditions) to decrease the rather high concentration of citrate. Immediately thereafter equilibrium dialysis was performed in the usual manner at pH 7.50 with the contents of the 3 flasks (containing fractions II III IV–V fractions IV V and fraction V respectively) The results are shown at the bottom of table 55 They are marred by the fact that obviously citrate was still present in significant amounts ((cat min Cl + HCO_3) for dialysis solution 4.9 values for Δ Ca + Mg lower than expected). Furthermore, whereas the solution containing fractions IV–V and the solution containing fraction V were only slightly opalescent the solution containing fractions II III IV–V showed flocculation.

Nevertheless it would appear that from the results some conclusions can be drawn. Whereas in the case of fractions II–III IV–V (which should be very much like plasma) the values for NCE, Δ Na and Δ K are indeed of the same magnitude as found for normal serum, the values found for fractions IV–V and for fraction V are higher especially those for Δ Na and Δ K and – in the case of fraction V – also that for NCE. The electrophoresis strip showed that fractions IV V were composed of albumin, α_1, α_2 and β-globulin in about the same relative proportions as in serum, whereas fraction V contained albumin with some α_2-globulin and a trace of α_1-globulin. We must therefore conclude that either α_2-globulin has a NCE which per unit weight is about 8 times larger than the NCE found for albumin, or that during the final precipitation the net negative charge of albumin is considerably altered. The experiment should obviously have included equilibrium dialysis of the albumin obtained from fraction V but such as it is the experiment can serve to illustrate the need for further investigation on this point and for caution in the calculation of values for serum from values obtained with purified fractions.

Returning to the results obtained for albumin it is difficult to say whether the differences between the values found for the various preparations are real in view of the SE of the determination An exception must be made for the experiments with the Red Cross preparations in which the citrate content of the dialysis solution was of the order of 0 5 mM/L But even there a distinct difference is only found for Δ Ca + Mg, the NCE value remaining relatively unaffected The small number of experiments and the variety or preparations studied effectively prevents an accurate estimate of the pH effect for albumin and for γ-globulin although for albumin the relation with pH appears to be similar to that found for normal serum. Table 57 may serve to indicate the approximate values at pH 7 45 These were obtained by simply averaging all values available within the pH range 7 30–7 70 with the exception of

for NCE, Δ Na Δ K the experiment in which the serum was deproteinised

for Δ Ca + Mg the experiments in which a significant amount (> 0 1 mM/L) of citrate was present in the dialysis solution

In table 57 the average values have not been corrected for systematic errors and the values listed for normal serum therefore refer also to uncorrected results (For Δ K the normal value at pH 7 45 was estimated from fig. 50)

When we attempt to correct for the systematic errors in NCE and Δ Na, we meet with the difficulty that in the case of the purified protein fractions the flame photometer error is not known for certain. The evidence presented in fig. 12 (chapter III) suggests that in the case of albumin the necessary correction may be about 0.8 mEq/100 g protein, and i the case of γ-globulin zero. But this conclusion is only based on one experiment and in equilibrium dialysis experiments with albumin comparison of the distribution of ^{22}Na (as measured with a scintillation counter) with that of ^{2} Na (as measured by flame photometry) suggested that in the case of albumin the flame photometer error is not larger than in the case of serum. No ashing experiments were done with salt solutions containing albumin or γ-globulin.

From the evidence available at present we can only estimate that in the case of Δ Na and of NCE the correction for the flame photometer error is somewhere between 0.2 and 0.8 mEq/100 g albumin and between 0.0 and 0.2 mEq/100 g γ-globulin. In the case of NCE the correction for carbamino-protein should be added, which we have assumed to be equal to the correction accepted for normal serum, *i.e.* 0.3 mEq/100 g albumin or γ-globulin. According to this reasoning, the corrected values for NCE should therefore be 20.2 to 20.8 mEq/100 g albumin and −1.9 to −1.7 mEq/100 g γ-globulin. The magnitude of the correction remains small and does not affect the conclusions concerning the different behaviour of albumin and γ-globulin and the relatively low value of NCE in the case of albumin.

In comparing the values given for Δ Na, Δ K and Δ Ca + Mg it should once again be kept in mind that they are not corrected for the effect of the Donnan equilibrium. Since the latter depends on net charge the differences between the values obtained for albumin and for γ-globulin will in reality be larger.

Concentration ratios

These are presented in table 55, but we will only consider R_X and R_{Cl} in more detail. To facilitate comparison with the results obtained for normal serum we have averaged our results for the two pH ranges 6.60–7.20 and 7.20–7.70, as in table 52 for normal sera. (All experiments have been included with the exception of the one in which Na was determined after deproteinisation).

Table 58 summarises our efforts. The effect on R_X of the correction for the estimated flame photometer error discussed in the previous paragraph is indicated. In the case of albumin and γ-globulin we have not attempted to calculate the SE of the mean as we are dealing here with fractions prepared from different sources and by different methods. But even so it would appear that for albumin R_X is definitely lower and R_{Cl} somewhat higher than for normal serum. Since for albumin the negative net charge is certainly somewhat larger per unit weight than for the proteins as present in serum, this discrepancy between the concentration ratios of the two oppositely charged ions is compatible with the assumption that albumin binds per unit weight a larger amount of Cl in a complex-type manner than the other serum proteins. This explains to some extent the fact that the difference between serum and albumin as regards NCE is less than the differences found for Δ Na and Δ Ca + Mg would lead us to expect.

For γ-globulin the number of samples is so small that no definite conclusion is possible whether the difference between R and R_{Cl} is a real one here we cannot decide. But the fact that they are both higher than 1.000 is another indication that the net charge of γ-globulin is positive in the pH range studied and gives even more significance to the fact that R_{Cl} is still 0.84 at pH 7.47 and 0.91 at pH 6.82 (see table 55).

Comparison and discussion of the results obtained by means of in vivo ultrafiltration and by means of equilibrium dialysis

We will first consider the values obtained for NCE, then the values obtained for protein bound cation and finally we will try to estimate values for the Donnan ratio and for the amounts of ion bound in a salt type respectively complex type manner

NET CATION EQUIVALENCY (NCE)

RESULTS OBTAINED FOR NORMAL SUBJECTS

Table 59 compares the mean value of NCE obtained by means of *in vivo* ultrafiltration (table 42, chapter VI) with that obtained by means of equilibrium dialysis of serum samples (table 48 chapter VII) When we assume the buffer value to be 10 3 mEq/100 g protein/unit pH (see below) we find that at pH 7.34 the difference between the two mean values is 0 4 mEq/100 g protein. For the *not corrected* values of NCE in the 28 *in vivo* ultrafiltration experiments the SE of the mean was calculated at 0.2 mEq/100 g protein (see Addendum) Since a correction for systematic errors increases the accidental error we can conclude that the difference between the two mean values given in table 59 *is not statistically significant.*

In the case of the 9 *in vivo* ultrafiltration experiments at the end of which serum was obtained for dialysis the results can be compared with those obtained in the paired dialysis experiments (table 60) After correction for systematic errors the mean value of NCE is somewhat lower for the *in vivo* ultrafiltration series but again the difference is too small to be significant The latter conclusion is confirmed when the pairs of experiments are considered separately (last column of table 60)

The influence of differences in pH was eliminated by calculating for the dialysis experiments the NCE value to be expected at the pH of the corresponding *in vivo* ultrafiltration experiments assuming a buffer value of 10.3 mEq/100 g protein/unit pH

When we add to the 9 pairs of experiments in which serum was dialysed the 4 pairs of experiments in which *plasma* was dialysed, the mean value of NCE becomes 17.3 (at pH 7 35) in the case of the *in vivo* ultrafiltration experiments against 18 0 (at pH 7.34) in the case of the dialysis experiments. The difference is now somewhat larger due to the fact that for the dialysis experiments the mean value has become somewhat higher Although not statistically significant, the observation is compatible with the existence of a small positive error in NCE due to the amount of heparin present in the plasma samples.

When we average the two mean values of NCE presented in table 59 while taking into account the number of subjects studied in each of the two series of experiments and the

observed relation between pH and NCE (see below), we obtain the following estimate for the average value of the NCE of normal plasma proteins

NCE = 17.4 mEq/100 g protein (at pH 7.35)

or

NCE = 12.2 mEq/L plasma (at pH 7.35)

when we consider normal plasma to contain 70 g protein per litre.

The fact that for the *in vivo* ultrafiltration experiments the 'weighed' mean of NCE values not corrected for systematic errors (presented in the Addendum) is 0.2 mEq/100 g lower than their arithmetic mean value, on which the corrected mean value given in table 59 is based, indicates that by neglecting the different 'weight' of the individual *in vivo* ultrafiltration experiments we overestimate the mean value of NCE by about 1 % at most.

For the calculation of the correlation between pH and NCE the results of the dialysis experiments are available. If we adjust the equations derived in chapter VII to the value accepted as the best estimate of NCE for normal plasma proteins at pH 7.34 (see above) we obtain the following two equations
for the pH range 6.60 to 7.00

$$\text{NCE (mEq/100 g protein)} = 10.0\,(\text{pH} - 5.64)$$

for the pH range 7.00 to 7.70

$$\text{NCE (mEq/100 g protein)} = 10.7\,(\text{pH} - 5.72).$$

The reader is reminded of the fact that the difference between the two equations is due to the assumption that carbamino-protein is present at pH values above 7.00

The results of our experiments indicate that in the pH range observed in clinical medicine a reasonable estimate of the value of NCE at a given pH can be obtained from the following equation

$$\boxed{\text{NCE (mEq/100 g protein)} = 10.3\,(\text{pH} - 5.66)}$$

provided the composition of plasma proteins is normal and the concentrations of the plasma electrolytes do not differ much from normal.

We have not calculated the SE for the mean values given above. The correction for systematic errors precludes this. However the magnitude of the spread observed in our experiments is indicated by the fact that, for both methods used and at any given pH all experimental values for NCE were within ± 4 mEq of the non-corrected mean value calculated for that particular pH value.

RESULTS OBTAINED FOR PATIENTS AND RESULTS OBTAINED WITH PURIFIED PROTEIN FRACTIONS

Our observations suggest that it is unlikely that factors other than pH and protein composition have an important effect on the magnitude of NCE (expressed per 100 g protein).

Comparison and discussion of the results obtained by means of in vivo ultrafiltration and by means of equilibrium dialysis

We will first consider the values obtained for NCE, then the values obtained for protein-bound cation and finally we will try to estimate values for the Donnan ratio and for the amounts of ion bound in a salt type respectively complex type manner.

NET CATION EQUIVALENCY (NCE)

RESULTS OBTAINED FOR NORMAL SUBJECTS

Table 59 compares the mean value of NCE obtained by means of *in vivo* ultrafiltration (table 42, chapter VI) with that obtained by means of equilibrium dialysis of serum samples (table 48, chapter VII). When we assume the buffer value to be 10.3 mEq/100 g protein/unit pH (see below), we find that at pH 7.34 the difference between the two mean values is 0.4 mEq/100 g protein. For the *not corrected* values of NCE in the 28 *in vivo* ultrafiltration experiments the SE of the mean was calculated at 0.2 mEq/100 g protein (see Addendum). Since a correction for systematic errors increases the accidental error we can conclude that the difference between the two mean values given in table 59 is not statistically significant.

In the case of the 9 *in vivo* ultrafiltration experiments at the end of which serum was obtained for dialysis, the results can be compared with those obtained in the paired dialysis experiments (table 60). After correction for systematic errors the mean value of NCE is somewhat lower for the *in vivo* ultrafiltration series, but again the difference is too small to be significant. The latter conclusion is confirmed when the pairs of experiments are considered separately (last column of table 60).

The influence of differences in pH was eliminated by calculating for the dialysis experiments the NCE value to be expected at the pH of the corresponding *in vivo* ultrafiltration experiments assuming a buffer value of 10.3 mEq/100 g protein/unit pH.

When we add to the 9 pairs of experiments in which serum was dialysed the 4 pairs of experiments in which plasma was dialysed, the mean value of NCE becomes 17.3 (at pH 7.35) in the case of the *in vivo* ultrafiltration experiments against 18.0 (at pH 7.34) in the case of the dialysis experiments. The difference is now somewhat larger due to the fact that for the dialysis experiments the mean value has become somewhat higher. Although not statistically significant, the observation is compatible with the existence of a small positive error in NCE due to the amount of heparin present in the plasma samples.

When we average the two mean values of NCE presented in table 59 while taking into account the number of subjects studied in each of the two series of experiments and the

in which the pH was within the range 7 10-7.50 The Na value is corrected for the error due to flame photometry (table 50 in chapter VII). Finally we have listed the values obtained after correcting the results of the dialysis experiments for the Donnan effect. The manner in which the latter correction was made needs some explanation.

In chapter I it was shown that in order to obtai the total amount of cation bound in one way or other by protein an amount equal to $(1 - r)$ $[Cat]_s$ (for bivalent cations $(1 - r^2)$ $[Cat]_s$) must be added to Δ Cat. I these equations both Δ Cat and r refer to the actual protein concentration found in the experiments. From evidence presented in the third part of this chapter we can estimat the Donnan ratio to be 0.964 at pH 7.33 for normal serum with protein concentration of 70 g/Kg H_2O For the 14 dialysis experiments from which the data in table 61 were derived the verage protein concentration was 62.3 g/Kg H_2O and since the relation between protein concentration and is practically linear one for the electrolyte concentrations in our experiments (see chapter I) we can estimate the average value of t be 0 968 at the protein concentration observed in our experiments. From this, and from $[Cat]_s$ we calculate that the concentration of filtrable cation opposing filtrable anion in inside the dialysis unit lower the outside by 4.6 mEq/Kg H_2O in the case of Na, and by 0.26 mEq/Kg H_2O in the case of Ca + Mg. These values must therefore be subtracted from $[Cat]_s$ to obtain the concentration of 'free' cation i the serum *i.e.* cation not bound by protein in one way or other Conversely to obtain the total amount of cation protein-bound at the protein concentration of 62 3 g/Kg H_2O the bove values must be added to the values of Δ Cat actually found. The values thus corrected must still be multiplied by 100/62.3 to obtain the value for Δ Cat in mEq per 100 g of protein.

In this way the values listed on the last line of table 61 were calculated Although an approximation they can serve to indicate the magnitude of the Donnan effect.

If we compare the corrected dialysis results with the result of the *in vivo* ultrafiltration experiments we find that the latter are about 4 8 mEq/100 g protein lower in the case of protein-bound Na and about 0 15 mEq/100 g protein lower in the case of protein-bound Ca + Mg. In view of the fact that we found nearly the same value for NCE with both methods we can assume that the differences observed for protein bound Na and for protein-bound Ca + Mg are indeed due to neglect of the change in Donnan ratio occurring during haemoconcentration It should be noted that these differences are of the same magnitude as the error estimated in chapter IV from the trend of the sum concentration of Cl + HCO_3 during haemoconcentration assuming 2 mEq of Cl to be complex-bound by the albumin normally present in one litre of plasma. This finding supports the assumption that in plasma a certain amount of anion is bound in a complex type manner by protein. It is also compatible with a Donnan ratio of about 0.97 at the capillary membrane (when pH and protein concentration are normal).

For K no values are given in table 61 In the *in vivo* ultrafiltration experiments no reliable results could be obtained and the amount calculated from the dialysis experiments is d bious because of the relatively large correction necessary for the flame photometer error But on the average about 0 3 to 0.4 mEq of K must have been bound per 100 g normal plasma protein at normal pH values.

We can make an estimate of the influence of pH on the total amount of protein-bound Na and protein-bound Ca + Mg by correcting the experimental relation found for Δ Na, and for Δ Ca + Mg with the aid of the values which the Donnan ratio is estimated t have at pH 6.91 and 7 46 (third part of this chapter) while using the same approach as described above in connection with table 61

After correcting for flame photometer error and for the Donnan effect, the following

The effect of pH is evident from the discussion in the previous section. As a result one can expect the value of NCE to vary from about 8 mEq/100 g protein at pH 6.90 to a value of about 23 mEq/100 g protein at pH 7 70 – the pH values mentioned representing the extremes of the clinical pH range

As to protein composition the results both of the *in vivo* ultrafiltration experiments and of the dialysis experiments with pathological sera and purified protein fractions indicate that for normal γ globulin and for the paraprotein(s) of γ-myeloma the NCE is of the order of 2-6 mEq/100 g protein at a pH value of 7.35 For the other fractions our results are more equivocal but it seems reasonable to conclude that *in vivo* albumin and at least some of the α and possibly the β-globulins have a NCE approximately 10 to 20 / higher than that found for the normal mixture of plasma proteins, the value being on the average 20–21 mEq/100 g protein at a pH value of 7 35 This is a surprisingly low value for albumin preliminary experiments in which fractions obtained with the cold-ethanol method 6 were compared (chapter VII) seem to indicate that in the process of precipitation and further purification the NCE of albumin decreases. Another possibility is that some of the α and β-globulins have an excessively high NCE (40–100 mEq/100 g protein)

In the *in vivo* ultrafiltration experiments it was observed that the β-type of myeloma is different from the γ type in that its paraproteins have a much higher NCE. But the fact that with equilibrium dialysis the serum proteins of one patient with a γ-type of myeloma were found to have a nearly normal value for NCE indicates that the paper electrophoretic pattern is not an infallible guide where the magnitude of NCE is concerned

Finally it must be stressed that even in patients with severe uraemia NCE of the plasma proteins was in the range expected when the effect of pH and protein composition was taken into account. This is interesting in view of the fact that in these patients relatively large amounts of organic substances are present in plasma. In the 3 patients with hypercholesterolaemia also a normal value was observed for NCE.

QUANTITY OF PROTEIN BOUND CATION

RESULTS OBTAINED FOR NORMAL SUBJECTS

The values calculated from the *in vivo* ultrafiltration experiments and from the dialysis experiments are both underestimates because of the effect of the Donnan equilibrium. A correction can be made if the Donnan ratio is known In the third part of this chapter we will make an attempt to estimate from our dialysis experiments average values for the Donnan ratio at different pH values. Since we possess no direct information concerning the composition of interstitial fluid no correction is possible for the results obtained with *in vivo* ultrafiltration But by comparing the latter with the values found when the results of the dialysis experiments are corrected for the Donnan effect, some conclusions concerning the Donnan ratio at the capillary membrane should be possible.

In table 61 we have first listed the results of the *in vivo* ultrafiltration experiments as presented in table 41 (chapter VI) The values are those obtained after correction for systematic errors (with the exception, of course, of the error assumed for the Donnan effect) Below these are listed the results obtained for serum in the 14 dialysis experiments

in chapter VII. If an approximate correction is made for the Donnan effect with the aid of the Donnan ratios estimated in the third part of this chapter we arrive at the conclusion that at pH 7 40 total albumin-bound Na is about 22 mEq/100 g albumin, whereas at the same pH the amount of Na bound by γ-globulin is negligible, or even negative – indicating that in the latter case protein net charge must be positive In a similar manner we can estimate that at pH 7 40 total protein-bound Ca + Mg is approximately 5 0 mEq/100 g albumin and about 1 0 mEq/100 g γ-globulin when the concentration of 'free cation is about 2.6 mEq/Kg H_2O for Ca, and 1.3 mEq/Kg H_2O for Mg.

The behaviour of K towards albumin and γ-globulin is qualitatively the same as that of Na.

THE DONNAN RATIO AND THE NATURE OF ION-PROTEIN BINDING NEGATIVE NET CHARGE OF PROTEIN

In chapter I we argued that in dialysis experiments such as ours the Donnan ratio can be obtained either from the concentration ratio of a given cation (or anion) when the magnitude of its complex-bound fraction is known, or from the concentration of filtrable anion and the protein net charge in the serum But neither approach is possible in our case since we determine NCE, not protein net charge The latter can only be calculated from the first if the total amounts of both complex-bound cation and anion are known and for these we must know the Donnan ratio We are therefore caught in a cleft stick. However we can try how far reasoning will lead us.

NORMAL SERUM

Estimation of Donnan ratio

In chapter I it was shown that Cl is the only one of the ions under consideration for which a reasonable estimate can be made of the quantity which at physiological pH is complex-bound by albumin. Furthermore, evidence was presented which indicates that at pH 7 00 the amount of Na bound a complex-type manner by albumin must be negligible, *i.e* presumably less than 2 of the Na present in the solution

In our dialysis experiments we found, both with serum and with albumin and for the whole pH range studied, R_{Na} lower than R_{Cl} The difference appears to be real since it was found in every single experiment. The difference is the more pronounced the lower the pH. Therefore, one or both of these ions must be partially complex bound by protein The fact that both for serum and for albumin the average value of R_{Cl} was found very nearly 1 000 at a pH where NCE is still about 10 mEq per 100 g of protein suggests that around pH 7.00 it must be mainly Cl which is bound in a complex-type manner Qualitatively therefore both our findings for normal serum and for albumin are in agreement with the experimental results obtained by SCATCHARD *et al.* and by CARR, who studied purified albumin and used a different technique.

In order to interpret our data on serum in a more quantitative way we will first consider the *results obtained th albumin* We assume that at pH 6.80 (the average pH of the low pH range in our experiments with albumin – see table 58 in chapter VII) no complex-bound Na exists. In that case $R_{Na} = r$ With the aid of equation (9) in chapter I

relationship between protein bound Na and pH is found

$$\text{protein bound Na (mEq/100 g)} = 7\,3\,(\text{pH} - 5\,10)$$

In the case of protein-bound Ca + Mg the *correction for Donnan effect somewhat* increases the curvilinearity of the relation with pH But since for the pH range 7 00 to 7 50 the *relation is roughly* rectilinear we find after correction for the Donnan effect

$$\text{protein bound Ca + Mg (mEq/100 g)} = 2.7\,(\text{pH} - 5\,87)$$

In our experiments the ratio between the concentrations of free Ca and free Mg was about 2 1 If we assume that the protein bound fraction is about equal for both ions in the pH range studied (see chapter VII) we can estimate the increase per unit pH to be for Ca 1 8 mEq/100 g *protein and for Mg* 0 9 *mEq/100 g protein*

RESULTS OBTAINED FOR PATIENTS AND RESULTS OBTAINED WITH PURIFIED PROTEIN FRACTIONS

The error due to the Donnan effect is considerable in the case of protein-bound Na but it is relatively small in the case of the bivalent cations because in the latter case most of the binding is of the complex *type and thus not affected* by the Donnan ratio For Ca and Mg some conclusions can therefore be drawn from *in vivo* ultrafiltration in *patients with abnormal plasma proteins, if allowance is made for differences in free* Ca and free Mg concentration On the whole the results obtained with *in vivo* ultra filtration and those obtained for the few pathological sera which were dialysed, are in good agreement with the results obtained when dialysing purified protein preparations. They all show that although Ca (and Mg) are certainly bound to a lesser extent by γ globulin than by albumin the difference is not so pronounced as for NCE. At physiological pH values γ globulin binds still relatively large amounts of bivalent cations although *its net charge is only just negative or even slightly positive*

While discussing the results obtained for NCE we have already mentioned the observation that the serum of one patient with an *apparently typical γ myeloma* had an unexpectedly high value of NCE. The same was found for Δ Na, but the difference was not nearly so marked for Δ Ca + Mg.

For the various albumin solutions studied by equilibrium dialysis the spread in Δ Na was remarkably small, much smaller indeed than the spread found for the NCE values. Furthermore since in the case of albumin both Δ Na and Δ Ca + Mg were found distinctly higher than in the case of normal serum the fact that the NCE values were *on the whole only slightly* higher than the values found for normal serum must indicate that more Cl (and HCO_3?) is complex bound by 100 g of albumin than by 100 g of *normally composed serum proteins.*

Because of the limited number of experiments we have made no attempt to derive equations for *the relation between* pH and NCE *respectively protein-bound cation in* the case of albumin and of γ-globulin but the general trend can be gathered from fig. 54

in chapter VII If an approximate correction is made for the Donnan effect with the aid of the Donnan ratios estimated in the third part of this chapter we arrive at the conclusion that at pH 7 40 total albumin-bound Na is about 22 mEq/100 g albumin, whereas at the same pH the amount of Na bound by γ-globulin is negligible, or even negative – indicating that in the latter case protein net charge must be positive In a similar manner we can estimate that at pH 7 40 total protein-bound Ca + Mg is approximately 5 0 mEq/100 g albumin and about 1.0 mEq/100 g γ-globulin when the concentration of 'free cation is about 2.6 mEq/Kg H_2O for Ca, and 1.3 mEq/Kg H_2O for Mg.

The behaviour of K towards albumin and γ-globulin is qualitatively the same as that of Na.

THE DONNAN RATIO AND THE NATURE OF ION PROTEIN BINDING – NEGATIVE NET CHARGE OF PROTEIN

In chapter I we argued that in dialysis experiments such as ours the Donnan ratio can be obtained either from the concentration ratio of a given cation (or anion) when the magnitude of its complex-bound fraction is known, or from the concentration of filtrable anion and the protein net charge in the serum. But neither approach is possible in our case since we determine NCE, not protein net charge. The latter can only be calculated from the first if the total amounts of both complex-bound cation and anion are known and for these we must know the Donnan ratio We are therefore caught in a cleft stick. However we can try how far reasoning will lead us.

NORMAL SERUM

Estimation of Donnan ratio

In chapter I it was shown that Cl is the only one of the ions under consideration for which a reasonable estimate can be made of the quantity which at physiological pH is complex bound by albumin. Furthermore, evidence was presented which indicates that at pH 7 00 the amount of Na bound in a complex-type manner by albumin must be negligible, *i.e* presumably less than 2 of the Na present in the solution.

In our dialysis experiments we found both with serum and with albumin and for the whole pH range studied, R_{Na} lower than R_{Cl} The difference appears to be real since it was found in every single experiment. The difference is the more pronounced the lower the pH. Therefore, one or both of these ions must be partially complex-bound by protein The fact that both for serum and for albumin the average value of R_{Cl} was found very nearly 1 000 at a pH where NCE is still about 10 mEq per 100 g of protein suggests that around pH 7 00 it must be mainly Cl which is bound in a complex-type manner Qualitatively therefore both our findings for normal serum and for albumin are in agreement with the experimental results obtained by SCATCHARD *et al* and by CARR, who studied purified albumin and used a different technique.

In order to interpret our data on serum in a more quantitative way we will first consider the *results obtained with albumin* We assume that at pH 6.80 (the average pH of the low pH range in our experiments with albumin – see table 58 in chapter VII) no complex-bound N exists. In that case R_N = r With the aid of equation (9) in chapter I,

we calculate then from table 58 (using the lowest of the two values *given for corrected* R_{Na})

complex bound Cl = 5 7 mEq/Kg H_2O at an albumin concentration of 70 g/Kg H_2O

Thus we find that at pH 6 80 and at a Cl concentration of about 130 mEq/Kg H_2O 5 7 moles Cl are bound per mole albumin (mol weight ~70 000) This should be the maximum value since it was calculated on the assumption that no Na is complex bound. If 1 % of the Na present in the albumin solution were complex bound, the Donnan ratio would be 0 964 (equation (3) in chapter I) and in that case we calculate that 4 6 moles Cl are bound per *mole albumin In a similar manner we calculate from the experi*ments in the high pH range (table 58) that, at pH 7 44 4 6 moles Cl are complex *bound per mole albumin* if no Na is complex bound, and that 3 4 moles Cl must be complex bound per mole albumin if 1 % of Na is bound in a complex type manner

In chapter I we came to the conclusion that the data from the literature indicate that at pH 7 40 a value between 0 5 and 3 0 is the most reasonable estimate for the moles of Cl that are complex bound per mole albumin The values calculated above are *somewhat higher – especially the one calculated on the assumption that no Na is bound* in a complex type manner In this connection it must be remembered that the results obtained by SCATCHARD *et al* by VAN OS & KOOPMAN–VAN EUPEN and by CARR do not exclude the possibility that 1–2 % of Na is complex-bound at our experimental conditions.

If we now return to our main interest which is *plasma under physiological conditions* we cannot neglect the observation of Carr that α and β-globulins bind Na in a complex type *manner* His *results appear to indicate that, at pH 7.50* 2–3 mEq of Na are bound per 100 g of these proteins when the Na concentration in the solution is only 15–20 mEq/L. In *the case of serum therefore we cannot consider* R_x *to be equal to* r But since albumin is apparently the only one of the serum proteins to bind Cl in a complex type manner at physiological pH (chapter I), we can make an estimate of the Donnan ratio by first calculating the amount of complex bound Cl present in serum from the albumin concentration of the latter and from the amount of Cl bound per mole albumin as calculated from the dialysis experiments

In our experiments 60 % of serum protein was albumin (average value found by paper electro*phoresis* 60 % *that found with the method of Majoor* 58 %). *If we assume that the* relation between pH and complex-bound Cl is linear over the pH range 6.80 to 7.50, we can estimate the *concentration of complex-bound Cl in serum* at *pH* 6.91 and at pH 7 46 to be respectively 3.31 and 2.74 mEq/Kg H_2O when no Na is complex-bound by albumen, and respectively 2.67 and 2.03 mEq/Kg H_2O when 1 % of Na is complex-bound.

With the aid of equation (5) in chapter I and the data presented in table 52 (chapter VII) we then calculate the *following values for the Donnan ratio of serum*

	pH 6 91	*pH 7 46*
if no Na is complex-bound by albumin	0 9666	0 9555
if 1 % of Na is complex bound by albumin	0 9717	0.9614

A glance at the average values of R_x found for normal serum (table 53 in chapter VII) reveals that these are practically the same as the values calculated above for r on the assumption *that no* Na is complex-bound by albumin

However there are several reasons why we consider a higher value for the Donnan ratio more probable especially in the high pH range. The first is the observation by CARR, already quoted, which suggests that at pH 7.50 α and β-globulins bind sufficient Na in a complex-type manner that the amount is not negligible at the concentrations in which these proteins are present in normal serum.

A second reason is the fact that our calculations are based on our experimental results for albumin and although the range of the individual values was similar to that found for serum, the number of experiments was considerably less. In addition to this we used the lower of the two values of corrected' R_{Na}, *i e* the one calculated on the assumption that albumin depresses the flame photometer reading to such an extent that an error of 0 6 mEq/70 g albumin results. It is quite possible that the error is smaller (see chapter VII). Finally if only 1 % of Na would have been complex-bound in our experiments with albumin, we calculate the Donnan ratio for serum already 0 005 higher

If we take into consideration all available evidence, we come to the following conclusion At pH 7 46 the value of r is probably somewhere between 0 960 and 0.965, whereas at pH 6.91 a value of about 0.970 seems the most reasonable estimate, since at the lower pH value there must be less complex bound Na respectively more complex bound Cl than at the higher pH value. We shall therefore assume that in our experiments with serum the average values were (at a protein concentration of 70 g/Kg H_2O)

r = 0.962	(at pH 7 46)
= 0.970	(at pH 6.91)

These values are somewhat arbitrary but they are not unreasonable. With some confidence it can be said that *the correct value for r must have lain between 0 955 and 0 967 t pH 7.46, and between 0 965 and 0 982 at pH 6 91* (the first value of each pair was calculated on the assumption that no Na is complex bound and the second value on the assumption that not more than 1 / of Cl is bound in a complex-type manner)

The amount of ion protein-bound in a salt-type respectively complex-type manner

We can now estimate for each ion the total amount protein-bound, as well as the fraction which s complex-bound, with the aid of equations (7) to (11) from chapter I inserting the alues given for Δ cation in chapter VII and the concentration ratios presented in table 5? and 53 (chapter VII).

> Δ Na was calculated from the equation relating Δ Na to pH given in chapter VII Δ K was estimated from fig. 50 in chapter VII Δ Ca + Mg was obtained by interpolation from table 51 (chapter VII)

The result is summarised in table 62. It can be seen that at the accepted values for r we calculate complex-bound Na to be less than 1 / of the Na present in solution, even at pH 7 46 At the latter pH value complex bound Cl is calculated at 1.5 / of total Cl concentration The values calculated for K and for HCO_3 are very much an approximation The relatively large and dubious systematic error and the fairly large accidental error in the case of K, and the even larger accidental error in the case of HCO_3 have already been mentioned. But table 62 suggests that for K the percentage which is com-

we calculate then from table 58 (using the lowest of the two values given for corrected R_{Na})

complex bound Cl = 5 7 mEq/Kg H_2O at an albumin concentration of 70 g/Kg H_2O

Thus we find that at pH 6 80 and at a Cl concentration of about 130 mEq/Kg H_2O 5 7 moles Cl are bound per mole albumin (*mol weight ~70 000) Thus should be the* maximum value since it was calculated on the assumption that no Na is complex bound. If 1 % of the Na present in the albumin solution were *complex bound the Donnan* ratio would be 0 964 (equation (3) in chapter I) and in that case we calculate that 4 6 moles Cl are bound per mole albumin *In a similar manner we calculate from the experiments* in the high pH range (table 58) that, at pH 7 44 4 6 moles Cl are complex *bound per mole albumin if no Na is complex bound and that 3 4 moles Cl must* be complex-bound per mole albumin if 1 % of Na is bound in a complex-type manner

In chapter I we came to the conclusion that the data from the literature indicate that at pH 7 40 a value between 0 5 and 3 0 is the most reasonable estimate for the moles *of Cl that are complex bound* per mole albumin The values calculated above are somewhat higher – especially the one calculated on the assumption that no Na is bound in *a complex type manner* In this connection it must be remembered that the results obtained by SCATCHARD *et al* by VAN OS & KOOPMAN–VAN EUPEN and by CARR do not exclude the possibility that 1–2 % of Na is complex bound at our experimental conditions

If we now return to our main interest which is *plasma under physiological conditions* we cannot neglect the observation of Carr that α and β-globulins bind Na in a complex type manner *His results appear to indicate that, at pH 7 50 2–3 mEq of Na are bound* per 100 g of these proteins when the Na concentration in the solution is only 15–20 mEq/L In the case of serum therefore we cannot consider R_{Na} to be equal to *r* But since albumin is apparently the only one of the serum proteins to bind Cl in a complex type manner at physiological pH (chapter I) we can make an estimate *of the Donnan* ratio by first calculating the amount of complex bound Cl present in serum from the albumin concentration of the latter and from the amount of Cl *bound per mole albumin* as calculated from the dialysis experiments.

In our experiments 60 % of serum protein was albumin (average value found by paper electrophoresis 60 % that found with the method of Majoor 58 %). If we assume that the relation between pH and complex-bound Cl is linear *over the pH range 6.80 to 7.50, we can estimate the* concentration of complex-bound Cl in serum at pH 6.91 and at pH 7 46 to be respectively 3 31 and 2.74 *mEq/Kg* H_2O when no Na is complex-bound by albumin, and respectively 2 67 and 2.03 mEq/Kg H_2O when 1 % of Na is complex-bound.

With the aid of *equation (5)* in chapter I *and the data presented* in table 52 (chapter VII) we then calculate the following values for the Donnan ratio of serum

	pH 6 91	*pH 7 46*
If no Na is complex bound by albumin	0 9666	0 9555
if 1 % of Na is complex-bound by albumin	0 9717	*0 9614*

A glance at the average values of R_{Na} found for normal serum (table 53 in chapter VII) reveals that these are practically the same as the values calculated above for r on the assumption that no Na is complex-bound by albumin

From table 62 we can calculate Σ [An]$_{t}$ by summating the concentrations found for Cl and HCO_3, adding 1 7 mEq for inorganic phosphate (see chapter V) and subtract the sum of complex-bound Cl, HCO_3 and inorganic phosphate to obtain [An]$_f$

c) Finally we can subtract from the value found for NCE (at 70 g/Kg H_2O protein) the difference between the sum of the quantities of Na, K, Ca and Mg and the sum of the quantities of Cl, HCO_3 and inorganic phosphate that are complex-bound by 70 g protein This should give us the negative net charge of the proteins (see chapter I). From the general equation for calculating NCE at a given pH value (first part of this chapter) we estimate NCE per 70 g protein to be 13 0 mEq at pH 7 46 and 8.9 mEq at pH 6.91 For the experiments on which table 52 (chapter VII) is based the average value of NCE (suitably corrected for flame photometer error and for the presence of carbamino-protein) was found to be 12.9 mEq at pH 7 46, and 8.8 mEq at pH 6 91 again per 70 g protein. In view of the fact that the general equation was derived from selected experiments and adjusted to fit both the values found with *in vivo* ultrafiltration and those found with dialysis at pH 7.35 the agreement is satisfactory We shall use here the average values of NCE found for the experiments represented in table 52.

In table 64 the values calculated for [Pr^-] are summarised Whether calculated according to *a*) or to *b*), identical values for [Pr^-] are found. This suggests that the equations (3) (5) and (6) in chapter I are consistent. When calculated according to *c*) the value of [Pr^-] is found somewhat higher at both pH values.

It must be realised that when we compare the values for [Pr^-] obtained according to) or to *b*) errors in determination play no role because exactly the same set of values is inserted in the different equations. This is not so for the third method since NCE was calculated from ([Na] + [K] + [Ca + Mg]) — ([Cl] + [HCO_3]) on the assumption that the concentration of any other cation or anion present was equal on both sides of the semipermeable membrane. Na, K, Ca, Mg, Cl and HCO_3 figure in table 52 (chapter VII) but the rest-anions do not. W could show that the assumption of equal distribution is correct for inorganic phosphate, which is the one other ion present in concentration higher than 1 mEq/L. But the assumption does not need to be true for the other anions present in serum. The non-esterified fatty acids present in normal serum in an amount of about 0 6 mEq/L are completely bound by protein. It is therefore probable that during the dialysis experiments their distribution was unequal. This may have resulted in over estimating NCE by about 0 5 mEq per 70 g protein. Since these organic anions must increase in concentration parallel with protein, similar error would be made in the *in vivo* ultrafiltration experiments (see chapter IV). However the differences between the values calculated for [Pr^-] in table 64 are small and it is practically impossible to say whether they are real or the result of cumulative accidental errors. If anything they indicate that we have made slight overestimate of NCE and not an underestimate.

If we assume values for which are 0 005 lower or higher than those on which table 64 is based, find that the absolute value of protein net charge is calculated about 1.5 mEq/70 g protein higher or lower but that the differences between the values calculated according to the three methods remain the same.

In table 65 the values obtained for the negative net charge are compared with those found for NCE. The values given for the negative net charge were calculated from the average of the values listed under *b*) and *c*) in table 61 The values given for NCE were calculated from the equation given in the first part of this chapter It can be seen that at the lower pH alue negative net charge is somewhat *higher* than NCE because at that pH and at the Donnan ratio indicated the total amount of complex-bound anion has become larger than the total amount of complex bound cation. From table 65 we

plex bound is probably at least as high as for Na. It suggests further that some of the HCO_3 present in serum behaves as if complex-bound At pH 7 46 this may be due to the fact that part of total CO_2 is not HCO_3 but carbamino-protein The accidental error is too large to allow the relation between pH and the average value of R_{HCO_3} to be used for calculating the amount of carbamate present in serum But the conclusion that some carbamino-protein probably is present at pH 7 40 is supported by the experiments with albumin and γ-globulin (see later)

The data on which table 62 is based offer no information concerning Ca and Mg separately This information was obtained from the 4 dialysis experiments with plasma in which Ca + Mg as well as Ca was determined. The result is shown in table 63 Since the contribution of heparin to the non filtrable valencies must have been small (*i e* not more than 5 / of the contribution of the proteins) we have assumed that the Donnan ratio was the same for plasma and for serum.

It will be seen that for Ca + Mg the values calculated are practically the same as those given in table 62, at a pH value which is nearly identical This suggests that the values given in table 63 also apply for serum The protein bound fraction of the cation concentration is for Mg only slightly lower than for Ca. The same holds for the percentage of protein-bound ion which is complex bound

The percentage of inorganic phosphate which is complex-bound by 70 g normal serum protein can be estimated at 5 / (see evidence presented in chapters IV and V). At the concentration used in our experiments this represents about 0 1 mEq of inorganic phosphate per 70 g protein, and this quantity has therefore been accepted in the calculation of the negative net charge of the serum proteins (see below).

In view of its key position in the calculation of the protein-bound quantity of any ion we have to consider the effect of an error in the estimation of r If r were 0 005 lower respectively higher than the average values accepted above the calculated amount of complex bound Ca + Mg would not change significantly but for Na either no complex bound fraction would exist (when r is 0 005 lower) or it would be about 1 5 mEq/Kg H_2O (when r is 0 005 higher) for Cl we would calculate the complex bound quantity 0 6 mEq/Kg H_2O higher respectively the same amount lower than the quantities given in table 62.

Negative net charge of protein

Since we are now in possession of approximative values for the amounts of complex bound cation and complex bound anion we can attempt to estimate the value of protein negative net charge (Pr^-). This can be done in three ways.

a) We can summate the values found for protein bound Na, K Ca and Mg (salt type and complex type), summate the values found for complex bound Na, K Ca and Mg and subtract the latter from the first This should give us the amount of cation balancing the negative net charge of the serum proteins

b) If we assume the activity coefficient of the cations opposing protein charge to be the same as that of the other filtrable cations in solution we can rearrange equation (6) from chapter I to

$$[Pr^-] = \frac{[An^-]_i - r^2[An^-]_i}{r^2}$$

From table 62 we can calculate Σ [An]$_i$ by summating the concentrations found for Cl and HCO_3, adding 1.7 mEq for inorganic phosphate (see chapter V) and subtract the sum of complex-bound Cl, HCO_3 and inorganic phosphate to obtain [An$^-$]$_i$.

c) Finally we can subtract from the value found for NCE (at 70 g/Kg H_2O protein) the difference between the sum of the quantities of Na, K, Ca and Mg and the sum of the quantities of Cl, HCO_3 and inorganic phosphate that are complex bound by 70 g protein. This should give us the negative net charge of the proteins (see chapter I). From the general equation for calculating NCE at a given pH value (first part of this chapter) we estimate NCE per 70 g protein to be 13.0 mEq at pH 7.46 and 8.9 mEq at pH 6.91. For the experiments on which table 52 (chapter VII) is based the average value of NCE (suitably corrected for flame photometer error and for the presence of carbamino-protein) was found to be 12.9 mEq at pH 7.46, and 8.8 mEq at pH 6.91 again per 70 g protein. In view of the fact that the general equation was derived from selected experiments and adjusted to fit both the values found with *in vivo* ultrafiltration and those found with dialysis at pH 7.35, the agreement is satisfactory. We shall use here the average values of NCE found for the experiments represented in table 52.

In table 64 the values calculated for [Pr$^-$] are summarised. Whether calculated according to *a*) or to *b*), identical values for [Pr$^-$] are found. This suggests that the equations (3), (5) and (6) in chapter I are consistent. When calculated according to *c*) the value of [Pr$^-$] is found somewhat higher at both pH values.

It must be realised that when we compare the values for [Pr$^-$] obtained according to a) or to *b*) errors in determination play no role because exactly the same set of values is inserted in the different equations. This is not so for the third method since NCE was calculated from ([Na] + [K] + [Ca + Mg]) $-$ ([Cl] + [HCO_3]) on the assumption that the concentration of any other cation or anion present was equal on both sides of the semipermeable membrane. Na, K, Ca, Mg, Cl and HCO_3 figure in table 52 (chapter VII) but the rest-anions do not. We could show that the assumption of equal distribution is correct for inorganic phosphate, which is the one other ion present in a concentration higher than 1 mEq/L. But the assumption does not need to be true for the other anions present in serum. The non-esterified fatty acids present in normal serum in an amount of about 0.6 mEq/L are completely bound by protein. It is therefore probable that during the dialysis experiments their distribution was unequal. This may have resulted in over-estimating NCE by about 0.5 mEq per 70 g protein. Since these organic anions must increase in concentration parallel with protein, a similar error would be made in the *in vivo* ultrafiltration experiments (see chapter IV). However, the differences between the values calculated for [Pr$^-$] in table 64 are small and it is practically impossible to say whether they are real or the result of cumulative accidental errors. If anything they indicate that we have made a slight overestimate of NCE and not an underestimate.

If we assume values for which are 0.005 lower or higher than those on which table 64 is based, we find that the absolute value of protein net charge is calculated about 1.5 mEq/70 g protein higher or lower but that the differences between the values calculated according to the three methods remain the same.

In table 65 the values obtained for the negative net charge are compared with those found for NCE. The values given for the negative net charge were calculated from the average of the values listed under *b*) and *c*) in table 61. The values given for NCE were calculated from the equation given in the first part of this chapter. It can be seen that at the lower pH value negative net charge is somewhat *higher* than NCE because at that pH and at the Donnan ratio indicated the total amount of complex-bound anion has become larger than the total amount of complex-bound cation. From table 65 we

can derive the following rough approximation of the relation between pH and the negative net charge of the (normal) plasma proteins in the pH range 6.9 to 7 5

$$\text{negative net charge (mEq/100 g protein)} = 6\,9\,(\text{pH} - 4\,96)$$

Finally although the *in vivo* ultrafiltration experiments do not permit direct conclusions concerning the nature of ion protein binding, we have seen in chapter IV and again in the second part of this chapter that the abscence of a decrease in the sum concentration of Cl + HCO_3 during haemoconcentration is a further argument for the existence of complex bound Cl (and HCO_3?) in plasma under *in vivo* conditions

PATHOLOGICAL SERA AND PURIFIED PROTEIN FRACTIONS

In the previous paragraph it has become evident that we can – at best – make an estimate of the Donnan ratio in the case of normal serum The small number of pathological sera studied makes it impossible to reach any definite conclusion as to the Donnan ratio in these cases beyond the general one that the lower the albumin concentration and the higher the (normal or pathological) γ globulin concentration the more the Donnan ratio will approach a value of 1 000 This conclusion is not completely correct since in the serum from one patient with myeloma the paraprotein behaved electrophoretically as a γ-globulin and yet the Donnan ratio must have been somewhere near 0 98 in view of the values found for R_N and R_{Cl} But even in the sera where a value of nearly zero was found for NCE, the value found for R_{Ca+Mg} indicated that a considerable amount of the bivalent cations was bound in a complex type manner

We have already discussed the results obtained with the purified *albumin* fractions as a preliminary to estimating the Donnan ratio for normal serum. A comparison with the amount of complex bound Cl estimated from experiments done by others showed a fair agreement – especially if we assume that some Na (up to 1 / of total Na concentration) is complex bound also The number of experiments done with purified γ-*globulin* was very small indeed, but the average values found for R_N and R_{Cl} were both positive, indicating that in the pH range studied the net charge of the γ globulins must have been positive The difference between the two concentration ratios is definitely smaller than in the case of albumin and of normal serum, and presumably Cl is associated with γ-globulins in a salt type manner only

Both in the case of albumin and of γ-globulin we found R_{HCO_3} somewhat higher than R_{Cl} at pH 7 44 but somewhat lower at pH 6 80 (table 55 in chapter VII). The differences are smaller than those observed for normal serum, but the trend is the same Since it is improbable that more HCO_3 is complex bound at higher pH values, whereas on the other hand it is to be expected that the amount of carbamino-protein increases with increasing pH the above mentioned observation is compatible with the presence of some 0.2 mEq/Kg H_2O carbamino-protein in serum at normal pH

Chapter IX

Summing up

In this study the term 'binding' signifies attraction between the large (non-filtrable) proteins and the small (filtrable) inorganic ions regardless of the strength and the nature of the attracting forces. For practical reasons two types of binding are assumed to exist (chapter I): the *salt*-type in which the filtrable ions are fully dissociated but statistically balance the corresponding electrostatic charge of the ionic groups on the protein molecules, and the *complex* type in which the filtrable ions merge with the protein molecules in such a manner that they no longer contribute to the ionic activity of the surrounding fluid. This is a simplification, but in a complicated solution such as plasma we cannot at present distinguish experimentally between finer differences in the nature of ion-protein binding. The distinction in salt- and complex-type of binding has the advantage that it is based on the effect of protein on the activity of the filtrable ions. In membrane equilibria it is the activity ratio which is subject to the dictates of the Donnan equilibrium and when a filtrable ion is partly protein-bound in a complex type manner the concentration ratio of that ion will differ from the Donnan ratio, the latter being equal to the activity ratio. The discrepancy can be used to calculate the complex bound fraction of the particular ion (chapter I). For the present and under the conditions existing in plasma this indirect approach appears to give values for complex-bound ion which are more accurate than those obtained from direct measurement of ion activity. But the simplifying assumptions involved should be kept in mind (chapter I).

The net effect of all protein-bound ion present in plasma manifests itself in the contribution of the proteins to the balance between cationic and anionic valencies in the solution. This contribution was termed 'base binding power' by Van Slyke but is described more accurately by the term net cation equivalency (NCE). In chapter I the NCE of the proteins is defined as the difference between the concentrations of protein-bound filtrable cation and protein-bound filtrable anion. This includes both the ions bound in a salt-type manner and those bound in a complex-type manner. For practical reasons the negative net charge of the proteins is defined in terms of the amount of dissociated filtrable cation opposing it. NCE and negative net charge differ in magnitude when the concentrations of complex-bound cation and complex-bound anion are unequal.

In solutions such as plasma negative net charge must be calculated from NCE and the amounts of complex-bound cation respectively anion. NCE is determined indirectly by relating protein concentration to the 'gap' which is left when the sum of filtrable anionic valencies present in plasma is subtracted from the sum of filtrable cationic valencies. The assumption is that protein is the only non-filtrable substance in plasma which binds ions.

The determination of NCE requires a high degree of accuracy in the analyses of filtrable ions and protein Furthermore, since most proteins are more vulnerable than was previously recognised plasma should be studied in its native state or at least under conditions which imitate the *in vivo* state as closely as possible This includes the prevention of ageing

A study of the literature reveals (chapter II) that the value generally accepted for the NCE of the plasma proteins – 16 mEq/L plasma – is based on one publication by VAN SLYKE *et al* in 1928 Their values were obtained in experiments with albumin and globulin prepared by ammoniumsulfate fractionation, prolonged dialysis and subsequent drying from two samples of horse serum and one sample of pooled human serum

An attempt to estimate the NCE of the plasma proteins from published data on the ionic composition of normal plasma proved unrewarding mainly because of the surprisingly large spread in the values reported for the normal Na concentration and for normal total base Furthermore the number of investigations in which Na, K Ca, Mg as well as Cl and HCO_3 were determined from the same plasma or serum samples is rather limited

A similar conclusion was reached with regard to the published data concerning the distribution of the above mentioned ions across the capillary wall or an artificial semipermeable membrane We could find no recent study in which the concentration ratios were determined for both cations and anions For the Donnan ratio of the equilibrium between plasma and interstitial fluid apparently no value is available other than the one calculated from the base binding power found by Van Slyke *et al*



In the present study we have determined the NCE of the plasma proteins under conditions closely resembling those existing *in vivo* In addition we have made an attempt to estimate for the inorganic ions in plasma the fraction which is protein-bound in a complex type manner by determining their concentration ratios in equilibrium dialysis experiments It was thought that these data would allow us to make a reasonable estimate of the value of the Donnan ratio for the equilibrium that normally exists between plasma and interstitial fluid

The NCE of the plasma proteins was determined by correlating changes in protein concentration with concomitant changes in (cat min Cl + HCO_3), *i.e* the difference between the sum concentration of Na, K Ca, and Mg and the sum concentration of Cl and HCO_3, under conditions in which the concentration of the other anions present could be assumed to be constant To obtain samples with different protein concentrations two methods were used.

The first was *in vivo* ultrafiltration (GERBRANDY *et al* 1957) Haemoconcentration of venous forearm blood was induced by compressing the upper arm for up to 35 minutes at a pressure of about 100 mm Hg. Before, during and after compression blood samples were taken from a cubital vein In normal subjects the maximal percentage rise in plasma protein concentration was on the average 70 % NCE was calculated by correlating the protein concentration of the samples with the values found for (cat min Cl + HCO_3) and the concentration of the rest anions (RA) was obtained by extra

polating to protein concentration zero. In a similar manner values were obtained for protein-bound and 'free' cation.

The second method used was equilibrium dialysis. Samples of fresh serum or plasma were dialysed at 37.5°C against a synthetic interstitial fluid using a cellulose membrane. The pH value was varied by changing both HCO_3 concentration and P_{CO_2}. NCE was calculated by correlating the difference between the values found for (cat min Cl + HCO_3) inside and outside the membrane with the protein concentration inside. This was also done for the individual cations in order to obtain data concerning protein-bound cation. From the same experiments concentration ratios were calculated for Na, K, Ca + Mg, Cl and HCO_3.

Both NCE and protein-bound cation were expressed in mEq per 100 g protein in order to eliminate the effect of differences in protein concentration. For similar reasons all concentration ratios were calculated for a protein concentration of 70 g/Kg H_2O which is about the average of the protein concentrations observed in the dialysis or ultrafiltration experiments reported by others.

Quite a number of assumptions are involved in calculating NCE and protein-bound cation in the manner described. Moreover the summation of analytical data may seriously affect the accuracy of the values obtained especially in the case of NCE. Chapter III is therefore devoted to the discussion of the systematic and accidental errors of the methods used for chemical analysis. In this respect the macro-Kjeldahl, the biuret method and the flame photometer analysis must be mentioned in particular. The latter technique was found to give a rather large positive error for Na when deproteinised plasma is analysed but only a small negative error when plasma is analysed. The evidence presented in chapter III suggests that chemical analysis was sufficiently accurate to permit the determination of NCE in the manner explained above.

In chapter IV the technique of *in vivo* ultrafiltration is described together with the results of experiments devised to detect errors in the use of this method for the determination of NCE. It was found that – provided the arm is kept completely relaxed throughout the course of the experiment – the physico-chemical changes resulting from venous stasis during 45 minutes do not seriously affect the calculation of NCE in the manner described. The relationship between protein concentration and (cat *min* Cl + HCO_3) was found to be rectilinear over the protein range encountered in our experiments; the protein composition remained unaltered during haemoconcentration; no significant change was observed in inorganic phosphate or lactate content (at least not in subjects without anaemia); the change in pH remained sufficiently small to allow a correction to be made for its effect on NCE. In chapter V the technique of equilibrium dialysis is discussed and it appears that reasonably accurate values can be obtained for NCE and for the concentration ratios of the various ions studied. An exception must be made for the H-ions: the pH of the samples taken from the cellulose bags tended to be higher than the pH of the dialysis solution surrounding them. The difference was small but sufficient to prevent the use of the H-ion concentration ratio as an indicator of the Donnan ratio.

With regard to the determination of protein-bound cation it was concluded that in the case of Ca and Mg the systematic errors remain relatively small with both methods.

In the case of Na and K however the Donnan equilibrium is responsible for a large systematic error which can only be corrected for if the Donnan ratio is known. Even then the values obtained for protein-bound K remain unreliable in the case of *in vivo* ultrafiltration because of unpredictable changes in the concentration of free K

In chapter VI the results obtained with *In vivo* ultrafiltration are presented and the magnitude of the systematic errors is discussed 28 healthy men (22 to 42 yrs old) and 29 patients (both male and female, aged 25 to 87 yrs) were studied in the fasting state. The patients were selected because of an abnormal composition of the plasma proteins or an abnormality of the plasma ions

Chapter VII presents the results obtained by means of equilibrium dialysis at pH values ranging from 6 3 to 8 0 Serum in a few instances plasma, from 19 normal subjects (1 female) was dialysed 15 of whom had also been studied by *in vivo* ultrafiltration Only four pathological sera were dialysed. They were obtained from patients with myeloma and 1 patient with macroglobulinaemia. In addition a number of experiments were done with albumin and with γ globulin solutions

In chapter VIII the results obtained are compared and conclusions are drawn concerning the Donnan ratio and the amounts of ion bound in a complex-type manner With either method nearly the same average value was found for the NCE of the normal plasma or serum proteins at pH 7 35 Further evidence that the two methods give comparable results is found in the observation that the dialysis experiments with serum from patients with myeloma gave similar results as *in vivo* ultrafiltration in patients with the same disease The results obtained for protein bound cation were also similar when the systematic error resulting from the Donnan equilibrium was taken into account. Finally although the experiments were done in the course of one and a half year we could detect no seasonal changes (chapter VI and Addendum) The results obtained with the two methods have therefore been averaged and possible differences between true plasma and serum neglected.



Our results can be summarised as follows (the values given are those found after correction for systematic errors)

For the *normal* subjects the average value of NCE was 18 mEq/100 g protein at pH 7 40 The individual values were within 4 mEq of the average. When expressed per litre plasma the *normal range* for NCE was found to be 10–15 mEq/L with an average value of 12 5 mEq/L In all normal subjects NCE was lower than 24 mEq/100 g protein the value to be expected from the equation given by Van Slyke *et al* in 1928 and which can be written as

$$\text{NCE (mEq/100 g protein)} = 10\,4\,(\text{pH} - 5\,08).$$

The difference was observed over the whole of the pH range studied and consequently we found the same absolute change in NCE per unit pH change as did Van Slyke and coworkers. This is born out by the equation which describes our results in the pH range

6.60–7 70 for plasma or serum with a normal protein composition and normal concentrations for the inorganic ions

$$\text{NCE (mEq/100 g protein)} = 10.3\ (\text{pH} - 5.66).$$

Van Slyke *et al* and others with them, have tacitly assumed "base binding power" to be identical with the negative net charge of the proteins. This is erroneous (chapter I) but in the case of the plasma proteins the quantitative difference is not large at least not in the pH range observed in medicine. For reasons discussed in chapter VIII we estimate that at pH 7.40 the absolute value of the negative net charge of the plasma proteins is about 8 % lower than NCE. The discrepancy increases with increasing pH but becomes smaller at lower pH values and at pH 6.90 the negative net charge is even greater than NCE (about 5 %) A rough approximation of the correlation between pH and negative net charge of the normal plasma proteins in the pH range 6.9 to 7.5 is given by the equation

$$\text{negative net charge (mEq/100 g protein)} = 6.9\ (\text{pH} - 4\ 96).$$

The distinction between NCE and negative net charge is not an academic one since only the latter should be used in the equations describing the Donnan equilibrium. It must however be stressed that the values given for negative net charge have been estimated on the assumption that ion-protein interaction is either of the salt-type or of the complex-type. Therefore they do not necessarily correspond with the exact number of negatively charged univalent ionic groups on the proteins present in excess of positively charged groups (chapter I).

The above estimate of negative net charge was calculated on the assumption that in our dialysis experiments the Donnan ratio for the equilibrium between normal serum and dialysis solution was on the average 0.962 at pH 7 46 and 0.970 at pH 6.91 (protein concentration 70 g/Kg H_2O). These values were not determined directly but estimated from circumstantial evidence including besides our own experiments observations made by Scatchard *et al* and by Carr on purified albumin (chapter VIII). The fact that during the *in vivo* ultrafiltration experiments the concentration of $Cl + HCO_3$ remained constant suggests that in normal subjects the Donnan ratio for the equilibrium existing across the capillary wall is of the order of 0.97 (chapter IV). These values are decidedly higher than those previously estimated for the Donnan ratio (MANERY 1954). But it should be stressed that values reported for the Donnan ratio and for the concentration ratios of the different ions can only be compared correctly by taking into account differences in pH, in protein concentration, in protein composition and in the concentration of the filtrable ions.

With regard to the concentration ratios of the inorganic ions our results confirm the conclusion of Manery that there is a significant difference between the values obtained for the univalent cations on the one hand and those obtained for the univalent anions on the other This suggests that part of the univalent ions is bound in a complex-type manner by normal plasma proteins.

In the case of Na and Cl our results appear to be fairly accurate and comparison of the relationship between their respective concentration ratios and the pH strongly suggests that t pH 6.90 the plasma proteins bind a significant amount of Cl in a complex

type manner By taking into account the results obtained by Scatchard *et al.*, by Carr and by ourselves *with albumin solutions* we could estimate the amount of complex bound Cl to be about 2 mEq/L in normal plasma at a pH value of 7 40 From the experiments reported by Carr one must conclude that this Cl is attached *to albumin* Evidence presented by other workers and our own observations suggest that under physiological conditions the amount of Na which is protein bound in a complex type manner is at the highest 1 0 to 1 5 mEq/L plasma This is about 10 ⁄ of the total amount of protein-bound Na in normal plasma which we calculated at 12 mEq/L. Again evi *dence presented by Carr suggests that it are mainly α* and β-globulin and fibrinogen which are responsible for the complex type binding of Na

For K and HCO_3 our results are much more equivocal because of the relatively large accidental – and in the case of K systematic – errors. When corrected for the latter the concentration ratio of K is on the average somewhat lower than the ratio found for Na suggesting that relatively more K is bound in a complex type manner In the case of HCO_3 the concentration ratio tends to be higher than the Cl ratio at pH 7 40 but lower at pH 6 90 this observation is compatible with the presence of a small amount (about 0 2 mEq/L plasma) of carbamino-protein at the higher pH value.

As our main interest was in the univalent ions, Ca and Mg were determined separately in only 4 of the dialysis experiments. The results, together with those obtained by means of *in vivo* ultrafiltration indicate that for Mg the protein bound fraction is relatively smaller than for Ca, at least in normal plasma It is interesting to note that CARR (1955) and VAN OS & KOOPMAN-VAN EUPEN (1957) – both measuring complex bound ion – found no difference in the affinity of albumin for Ca and Mg whereas IRONS & PERKINS (1962) – who studied among other things the effect of albumin on the distribution of radioactive Ca and Mg isotopes in equilibrium dialysis – concluded that the affinity for Ca was decidedly higher than that for Mg From the 4 experiments in which we determined the concentration ratio both for Ca and for Mg it would appear that in *plasma the percentage of the total amount of protein bound ion which is complex* bound is nearly the same for Ca and Mg (about 75 ⁄) The correlation between total protein bound ion (in mEq/100 g protein) and pH was only studied for the sum of Ca and Mg. It was distinctly curvilinear the protein bound fraction increasing progressively with increasing pH (chapter VII). LOKEN *et al* (1960) in their ultracentrifugal analysis of protein bound and 'free Ca in human serum observed a *similar relationship in the* pH range 6 5–7 5 but in their case the correlation was practically linear from pH 7 0 upwards In the ultrafiltration experiments reported by TORIBARA *et al* (1957) and HOPKINS *et al* (1952) the correlation between protein bound Ca and pH appeared to be linear over the pH range 6 0–8 0 but the number of observations in which the pH value was lower than 7 4 was very small

We have expressed our normal values per unit weight of plasma protein with the restriction that the composition of the latter be normal This addition is necessary since the *relative* contribution of the various protein fractions to NCE etc. differs. VAN SLYKE *et al* in 1928 did not determine the NCE of serum but *separated the serum proteins in an* albumin and a globulin fraction determined NCE for each fraction and then calculated the NCE to be expected for total serum protein At pH 7 40 they found NCE to be 28 mEq/100 g albumin and 19 mEq/100 g globulin We use quotation marks to

stress the fact that the fractions studied by Van Slyke differ from the fractions isolated by means of electrophoresis. We have studied electrophoretically pure albumin and γ-globulin but no other fractions, and we can therefore not predict the NCE of total plasma protein. But the values which we obtained at pH 7 40 for the NCE of albumin (20 to 22 mEq/100 g) and that of γ globulin (—2 to 0 mEq/100 g) differ considerably from those found by Van Slyke *et al* for their albumin and globulin fractions. In fact if we consider the amounts of albumin and γ-globulin normally present in plasma and compare the NCE found for albumin and for γ-globulin with the NCE found for plasma protein we must conclude either that the NCE of the protein fractions studied by us has decreased during the process of fractionation or that the α- and/or the β-globulins have a much higher NCE than albumin. The normal value found for NCE in patients with a relatively high α and ,-globulin but a low albumin content of their plasma does agree with this conclusion (chapter VI). It is also in keeping with the observation of Carr that the α- and β-globulins bind Na (and K) in a complex-type manner

It must be concluded that an accurate prediction of the NCE of plasma protein from its (electrophoretic) composition is not possible. Roughly speaking the higher the albumin or the and β-globulin content of the plasma the higher NCE, the higher the γ-globulin content the lower NCE will be. But even this approximative estimate may lead one astray since proteins which in routine paper electrophoresis behave as γ globulins apparently can have a NCE which is about equal to that of albumin as we observed in one patient with myeloma (chapter VII)

The behaviour of the different ions in the presence of albumin and of γ-globulin leads to the following conclusions. Whereas at comparable pH values the difference between the concentration ratios was larger in the case of albumin than in the case of serum, no significant difference was observed in the experiments with γ-globulin But in the latter case both ratios were at pH 7 40 higher than 1 000, which indicates that at that pH value the net charge of γ-globulin is positive At the same pH however γ-globulin binds Ca and Mg in significant amounts – obviously in a complex-type manner The observation lends support to the opinion that part of the Ca and Mg binding by plasma proteins does not depend on electrostatic attraction

In the case of the patients the results obtained by means of *in vivo* ultrafiltration were less accurate because haemoconcentration was generally less pronounced and the number of blood samples smaller than in the experiments with normal subjects. Even so NCE and protein-bound cation expressed per 100 g protein, were within the normal range for nearly all patients including those with severe uraemia and the 2 patients with a β-type of myeloma. The exception were the 5 patients with a γ-type of myeloma for whom both NCE and proteinbound cation were found to be considerably lower than normal the one patient with a γ-type of myeloma and yet a normal value for NCE has already been mentioned. When the actual values of NCE per litre plasma are compared the conclusion is different because of the abnormally high or low plasma protein concentration symptomatic for certain disease states. In one patient with a γ-type of myeloma and a total protein concentration of 149 g/L plasma we found NCE 12 mEq/L plasma (which is normal) but 8 mEq/100 g protein (which is definitely too low). The reverse was observed in patients with hypoproteinaemia. But, whether expressed per unit volume of plasma or per unit weight of protein NCE was always found to have a positive value

type manner By taking into account the results obtained by Scatchard *et al* by Carr and by ourselves with albumin solutions we could estimate the amount of complex bound Cl to be about 2 mEq/L in normal plasma at a pH value of 7 40 From the experiments reported by Carr one must conclude that this Cl is attached to albumin. Evidence presented by other workers and our own observations suggest that under physiological conditions the amount of Na which is protein bound in a complex type manner is at the highest 1 0 to 1.5 mEq/L plasma. This is about 10 % of the total amount of protein bound Na in normal plasma which we calculated at 12 mEq/L. Again evidence presented by Carr suggests that it are mainly α and β-globulin and fibrinogen which are responsible for the complex type binding of Na

For K and HCO_3 our results are much more equivocal because of the relatively large accidental – and in the case of K systematic – errors When corrected for the latter the concentration ratio of K is on the average somewhat lower than the ratio found for Na suggesting that relatively more K is bound in a complex type manner In the case of HCO_3 the concentration ratio tends to be higher than the Cl ratio at pH 7 40 but lower at pH 6 90 this observation is compatible with the presence of a small amount (about 0.2 mEq/L plasma) of carbamino-protein at the higher pH value

As our main interest was in the univalent ions Ca and Mg were determined separately in only 4 of the dialysis experiments The results, together with those obtained by means of *in vivo* ultrafiltration indicate that for Mg the protein bound fraction is relatively smaller than for Ca, at least in normal plasma. It is interesting to note that CARR (1955) and VAN OS & KOOPMAN VAN EUPEN (1957) – both measuring complex-bound ion – found no difference in the affinity of albumin for Ca and Mg whereas IRONS & PERKINS (1962) – who studied among other things the effect of albumin on the distribution of radioactive Ca and Mg isotopes in equilibrium dialysis – concluded that the affinity for Ca was decidedly higher than that for Mg. From the 4 experiments in which we determined the concentration ratio both for Ca and for Mg it would appear that in plasma the percentage of the total amount of protein bound ion which is complex bound is nearly the same for Ca and Mg (about 75 %) The correlation between total protein bound ion (in mEq/100 g protein) and pH was only studied for the sum of Ca and Mg. It was distinctly curvilinear the protein bound fraction increasing progressively with increasing pH (chapter VII) LOKEN *et al* (1960) in their ultracentrifugal analysis of protein bound and free Ca in human serum observed a similar relationship in the pH range 6 5–7 5 but in their case the correlation was practically linear from pH 7 0 upwards. In the ultrafiltration experiments reported by TORIBARA *et al* (1957) and HOPKINS *et al* (1952) the correlation between protein bound Ca and pH appeared to be linear over the pH range 6 0–8 0 but the number of observations in which the pH value was lower than 7 4 was very small

We have expressed our normal values per unit weight of plasma protein with the restriction that the composition of the latter be normal This addition is necessary since the relative contribution of the various protein fractions to NCE etc. differs. VAN SLYKE *et al* in 1928 did not determine the NCE of serum but separated the serum proteins in an albumin and a globulin fraction determined NCE for each fraction and then calculated the NCE to be expected for total serum protein At pH 7 40 they found NCE to be 28 mEq/100 g albumin and 19 mEq/100 g globulin We use quotation marks to

stress the fact that the fractions studied by Van Slyke differ from the fractions isolated by means of electrophoresis. We have studied electrophoretically pure albumin and γ-globulin but no other fractions, and we can therefore not predict the NCE of total plasma protein. But the values which we obtained at pH 7.40 for the NCE of albumin (20 to 22 mEq/100 g) and that of γ-globulin (—2 to 0 mEq/100 g) differ considerably from those found by Van Slyke *et al* for their 'albumin' and 'globulin' fractions. In fact if we consider the amounts of albumin and γ-globulin normally present in plasma and compare the NCE found for albumin and for γ-globulin with the NCE found for plasma protein, we must conclude either that the NCE of the protein fractions studied by us has decreased during the process of fractionation or that the α- and/or the β-globulins have a much higher NCE than albumin. The normal value found for NCE in patients with a relatively high α- and β-globulin but a low albumin content of their plasma does agree with this conclusion (chapter VI). It is also in keeping with the observation of Carr that the α- and β-globulins bind Na (and K) in a complex type manner.

It must be concluded that an accurate prediction of the NCE of plasma protein from its (electrophoretic) composition is not possible. Roughly speaking the higher the albumin or the α- and β-globulin content of the plasma the higher NCE, the higher the γ-globulin content the lower NCE will be. But even this approximative estimate may lead one astray since proteins which in routine paper electrophoresis behave as γ-globulins apparently can have a NCE which is about equal to that of albumin as we observed in one patient with myeloma (chapter VII).

The behaviour of the different ions in the presence of albumin and of γ-globulin leads to the following conclusions. Whereas at comparable pH values the difference between the concentration ratios was larger in the case of albumin than in the case of serum, no significant difference was observed in the experiments with γ-globulin. But in the latter case both ratios were at pH 7.40 higher than 1.000, which indicates that at that pH value the net charge of γ-globulin is positive. At the same pH, however γ-globulin binds Ca and Mg in significant amounts – obviously in a complex-type manner. The observation lends support to the opinion that part of the Ca and Mg binding by plasma proteins does not depend on electrostatic attraction.

In the case of the patients the results obtained by means of *in vivo* ultrafiltration were less accurate because haemoconcentration was generally less pronounced and the number of blood samples smaller than in the experiments with normal subjects. Even so NCE and protein-bound cation, expressed per 100 g protein, were within the normal range for nearly all patients, including those with severe uraemia and the 2 patients with a β-type of myeloma. The exception were the 5 patients with a γ-type of myeloma for whom both NCE and proteinbound cation were found to be considerably lower than normal. The one patient with a γ-type of myeloma and yet a normal value for NCE has already been mentioned. When the actual values of NCE per litre plasma are compared the conclusion is different because of the abnormally high or low plasma protein concentration symptomatic for certain disease states. In one patient with a γ type of myeloma and a total protein concentration of 149 g/L plasma we found NCE 12 mEq/L plasma (which is normal) but 8 mEq/100 g protein (which is definitely too low). The reverse was observed in patients with hypoproteinaemia. But, whether expressed per unit volume of plasma or per unit weight of protein, NCE was always found to have a positive value.

in the physiological pH range even in the case of serum from patients with a γ type of myeloma the pH had to be lowered to 6 90 before the value of NCE decreased to zero We are therefore inclined to think that the transiently negative values for NCE observed by Broch in patients with fairly normal plasma pH and protein composition were due to errors in determination

The most intriguing of our results is the low value found for NCE in normal subjects. The difference with Van Slyke s value is too large to be explained by accidental errors and the evidence presented in previous chapters makes it unlikely that it is due to systematic errors in our determinations It is possible that in Van Slyke s experiments the fractionation and subsequent treatment of the proteins have increased their NCE by removing adhering substances such as fatty acids. Furthermore some of our observations suggest that prolonged storage of plasma or serum tends to increase the NCE of the proteins (chapter V) Still another explanation is suggested by the fact that during dialysis of the isolated protein fractions Van Slyke *et al* kept the pH of the solutions at about 5 0 so as to remove all traces of cation other than H ion Since it is now known that at pH values below 6 0 albumin binds a considerable amount of Cl in a complex type manner it is conceivable that whereas all Na had been removed the albumin used in the experiments of Van Slyke *et al* still contained some Cl In the final titration Cl concentration was not determined but calculated on the assumption that the weighed amount of NaCl added to the dialysed protein solutions accounted for all of the Cl present As the base binding power of the proteins was obtained by subtracting from the Na concentration the concentration of Cl + HCO_3, neglect of an extra quantity of Cl added with the albumin would lead to calculating erroneously high values for NCE. The high value found for globulin can partly be explained by the fact that the preparation studied by Van Slyke *et al* must have contained α and β-globulin besides γ globulin Another contribution to the calculation of erroneously high values for NCE may have come from a systematic negative error in the determination of protein concentration Van Slyke *et al* used a factor of 6 25 for converting nitrogen concentration into protein concentration but later studies by Van Slyke and co-workers (HILLER *et al* 1948) suggest that with the Kjeldahl method used in 1928 a nitrogen factor of 6 5 would have been more correct. The resulting error would account for one fifth of the difference between the NCE value found by Van Slyke *et al* and the value found by us. Although several possibilities suggest themselves we must conclude that we have no definite explanation for the difference observed



We can use our results to draw a picture of the ionic composition of normal plasma Table 66 shows our values for normal plasma (venous blood from the resting forearm) For the filtrable ions the values were taken from table 40 but NCE was calculated from the equation describing the correlation between pH and NCE. As a result the value of RA is 0 4 mEq/Kg H_2O higher than in table 42 the difference is acceptable since even for the non-corrected values of RA 2 SE of the mean is larger than that (Addendum) Table 66 should be compared with table 2 in which the results obtained by others are given Strictly speaking our values only apply to fasting men aged 20 to 40 yrs and they

TABLE 66 – *Average values for normal venous plasma (fasting state)*

pH	7.39
protein concentration	68.4 g/L (72.8 g/Kg H_2O)
protein composition	
albumin	60%
α_1-globulin	4%
α_2-globulin	8%
β-globulin	7%
fibrinogen	6%
γ-globulin	15%

cations		*anions*	
Na	139.0 (147.9)	Cl	106.0 (112.8)
K	4.0 (4.3)	HCO_3	26.1 (27.8)
		RA	5.1 (5.4)
Ca	4.7 (5.0)	inorg phosp	1.9
		lactate	1.4
Mg	1.7 (1.8)	NCE	12.2 (13.0)
sum	149.4 (159.0)	sum	149.4 (159.0)

The first value indicates the concentration in mEq/L plasma, the value between brackets the concentration in mEq/Kg H_2O.

may be somewhat different in the very young and the very old. We found the Na concentration somewhat lower and the Cl concentration somewhat higher than the average of the values quoted in table 2. Indeed our value for the normal Cl concentration is definitely higher, however, we studied fasting subjects and the observation by FAWCETT & WYNN (1956) that after a meal the plasma Cl concentration tends to decrease may explain the fact that our values are relatively high. Inorganic phosphate and lactate concentration were determined in part of the normal subjects only but when these values are subtracted from the 5.0 mEq/Kg H_2O actually calculated for RA from our *in vivo* ultrafiltration experiments we find that 1.5 mEq/L are left for SO_4 and the remaining organic acids. In view of the data quoted in table 3 this is not unreasonable. The pH value given in table 66 needs some comment. The average of the pH values actually measured was 7.36. But pH was determined at a temperature of 37.5°C and evidence presented in chapter IV strongly suggests that the temperature of the blood in the cubital veins is about 2 C lower. The average pH value was therefore corrected by adding 0.03.

Table 67 gives the Donnan ratio and the concentration ratios for the equilibrium between normal plasma and artificial interstitial fluid at a pH value of 7.40 and a temperature of 37.5°C. It was constructed from the evidence presented in chapters VII and VIII and should be compared with table 4. We found the ratio for Na considerably higher than the values previously reported, whereas no such difference was found for Cl. In view of the small spread in our individual values and in view of the special efforts to avoid systematic errors we believe our values to be more accurate than those given in table 4. In reality the distribution across the capillary wall will be slightly different

in the physiological pH range even in the case of serum from patients with a γ type of myeloma the pH had to be lowered to 6 90 before the value of NCE decreased to zero We are therefore inclined to think that the transiently negative values for NCE observed by Broch in patients with fairly normal plasma pH and protein composition were due to errors in determination

The most intriguing of our results is the low value found for NCE in normal subjects. The difference with Van Slyke s value is too large to be explained by accidental errors and the evidence presented in previous chapters makes it unlikely that it is due to systematic errors in our determinations. It is possible that in Van Slyke s experiments the fractionation and subsequent treatment of the proteins have increased their NCE by removing adhering substances such as fatty acids Furthermore some of our observations suggest that prolonged storage of plasma or serum tends to increase the NCE of the proteins (chapter V) Still another explanation is suggested by the fact that during dialysis of the isolated protein fractions Van Slyke *et al* kept the pH of the solutions at about 5 0 so as to remove all traces of cation other than H ion Since it is now known that at pH values below 6 0 albumin binds a considerable amount of Cl in a complex type manner it is conceivable that whereas all Na had been removed the albumin used in the experiments of Van Slyke *et al* still contained some Cl In the final titration Cl concentration was not determined but calculated on the assumption that the weighed amount of NaCl added to the dialysed protein solutions accounted for all of the Cl present. As the base binding power of the proteins was obtained by subtracting from the Na concentration the concentration of Cl + HCO_3, neglect of an extra quantity of Cl added with the albumin would lead to calculating erroneously high values for NCE. The high value found for globulin can partly be explained by the fact that the preparation studied by Van Slyke *et al* must have contained α and β-globulin besides γ globulin Another contribution to the calculation of erroneously high values for NCE may have come from a systematic negative error in the determination of protein concentration Van Slyke *et al* used a factor of 6.25 for converting nitrogen concentration into protein concentration but later studies by Van Slyke and co-workers (HILLER *et al* 1948) suggest that with the Kjeldahl method used in 1928 a nitrogen factor of 6 5 would have been more correct. The resulting error would account for one fifth of the difference between the NCE value found by Van Slyke *et al* and the value found by us. Although several possibilities suggest themselves we must conclude that we have no definite explanation for the difference observed



We can use our results to draw a picture of the ionic composition of normal plasma Table 66 shows our values for normal plasma (venous blood from the resting forearm) For the filtrable ions the values were taken from table 40, but NCE was calculated from the equation describing the correlation between pH and NCE. As a result the value of RA is 0 4 mEq/Kg H_2O higher than in table 42 the difference is acceptable since even for the non-corrected values of RA 2 SE of the mean is larger than that (Addendum) Table 66 should be compared with table 2 in which the results obtained by others are given Strictly speaking our values only apply to fasting men aged 20 to 40 yrs and they

In fig. 55 we have attempted to construct a cation-anion diagram for normal plasma after the manner of Gamble, allowing for the presence of complex-bound ions in the quantities estimated in chapter VIII. The latter are placed below the level of the gross horizontal line. Fig. 55 can be compared with D in fig. 7 (chapter I) by turning the latter upside down. Fig. 55 should be a reasonably accurate representation of the actual situation existing in plasma. However when systematic errors are made in one or more of the analyses of the individual ions the values given above for NCE and RA will not fit. This is demonstrated by the values obtained in the experiments in which flame photometer analysis was done on deproteinised plasma instead of plasma (chapter VI). This stresses once again the fact that we possess no direct determination of NCE. As a consequence all values reported, including ours, must be viewed with the disturbing effect of cumulative systematic errors in mind.

Our observations suggest that *in vivo* ultrafiltration can indeed be used for the determination of NCE in an individual patient with an accuracy of plus or minus 4 mEq/L plasma. Whether this will be a help in solving clinical problems remains to be seen but the finding of an unexpectedly high value for NCE in a patient with a γ type of myeloma suggests that also in this respect there is more to the plasma proteins than meets the eye at present. This is also true for the binding of the individual ions as demonstrated by the interesting observation of abnormal Ca binding reported by GLUECK *et al.* (1962). A distinct disadvantage of the method of *in vivo* ultrafiltration is the loss of blood involved which prevents at present frequent determinations in the same subject. The use of micro-methods for chemical analysis would considerably reduce the amount of plasma needed but the accuracy necessary is such that the introduction of a new analytical method necessitates a thorough search for systematic errors. The experience of others and of ourselves with the flame photometer analysis of plasma Na and K illustrates that it can be very difficult to exclude systematic errors with certainty. The problem of systematic errors also looms large in the determination of the amount of complex bound ion in plasma. We could only make an estimate by using circumstantial evidence. It will be interesting to see whether the specialised electrodes now coming into use can be made sufficiently selective to measure ion activity in plasma without systematic error. The problems involved are illustrated by the fact that it is still an open question whether the present glass-electrodes really measure H-ion activity.

Finally the question remains whether the NCE of the plasma proteins serves a biological purpose and has a regulatory function in the maintenance of the ionic composition of extracellular fluid. Contrary to Broch we find this rather difficult to believe. Whereas changes in the concentration of special protein fractions may conceivably contribute to the control of the level of free Ca or Mg, the NCE of the plasma proteins is the resultant of so many different protein-ion interactions that we fail to see how NCE *per se* can be part of a regulating mechanism. even as a buffer substance for H-ion concentration the plasma proteins are ineffective. Rather NCE should be considered a by-product of the fact that proteins are ionic substances. This does not mean that the determination of NCE is a waste of energy. The observation that it may differ considerably from the value expected from electrophoretic protein composition suggests that NCE is one of the characteristics by which plasma proteins can be differentiated. But

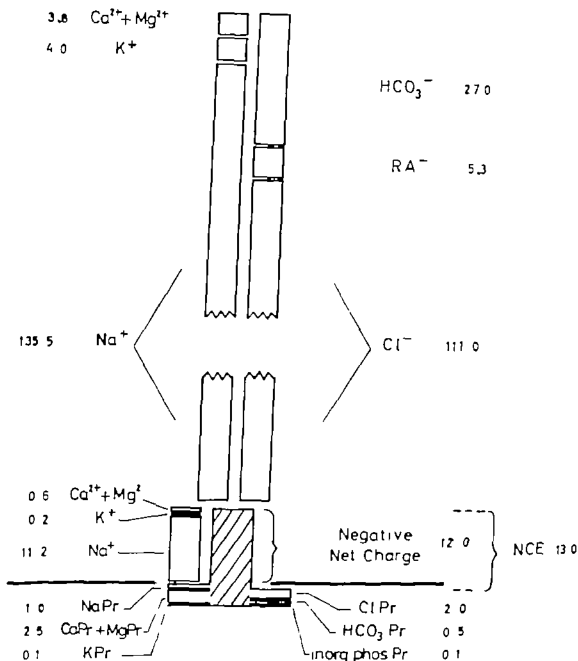


Fig. 55 Ionic composition of normal plasma (all values in mEq/Kg H_2O)

as a result of the presence of non filtrable ionic substances in the interstitial fluid The latter will increase all ratios but at present it is impossible to say how much

TABLE 67 – *Donnan ratio and concentration ratios for the equilibrium between plasma and artificial interstitial fluid at pH 7 40 and temperature 37.5 C plasma protein concentration 70 g/Kg H_2O protein composition normal*

r	R_{Ca}	R_{Mg}	R_K	R_N	R_{Cl}	R_{HCO_3}
0 963	0.57	0 65	0.94	0 956	0 979	0 99

List of Bibliographical References

ALBERTY, R. A. & H. H. MARVIN (1951) J. Amer. chem. Soc. 73, 3220.

ALDENHOVEN, H. & C. KORTH (1932) Deutsch. Arch. klin. Med. 173, 412.

ANDRES, R., G. CADER & K. L. ZIERLER (1956) J. clin. Invest. 35, 671.

ARNAUD, J. S. & A. S. RELMAN (1959) Amer. J. clin. Path. 31, 155.

ASTRUP, P. (1956) Scand. J. clin. Lab. Invest. 8, 33.

ATCHLEY, D. W., R. F. LOEB, E. M. BENEDICT & W. M. PALMA (1923) Arch. intern. Med. 31, 611.

ATCHLEY, D. W. & E. M. BENEDICT (1930) J. clin. Invest. 9, 265.

AUSPITZ, H. (1874) Vierteljahresschrift für Dermatologie und Syphilis 1, 275.

BALTZAN, M. A., R. ANDRES, G. CADER & K. L. ZIERLER (1962) J. clin. Invest. 41, 116.

BARKER, S. B. & W. H. SUMMERSON (1941) J. biol. Chem. 138, 535.

BATES, R. G. (1954) *Electrometric pH Determinations, Theory and Practice.* New York.

BENDIEN, W. M. & J. SNAPPER (1933) Biochem. Z. 260, 105.

BERGHOLD, H. (1932) Ergebn. inn. Med. und Kinderheilkunde, 42, 273.

BERGMAN, L., B. ISAKSSON & B. KÖÖHLER (1963) Lancet 1, 892.

BERGSTRÖM, J. (1962) Scand. J. clin. Lab. Invest. 14, Suppl. 68.

BERGSTRÖM, J. & E. HULTMAN (1962) Lancet 1, 1132.

BERNSTEIN, R. E. (1952) S. Afr. J. med. Sci. 17, 101.

BIEL, A. (1906) *Hypertonie als Heilmittel*, 4th ed. Leipzig.

BIRRELL, G. W. (1945) Amer. J. med. Sci. 210, 195.

BLACKFAN, K. D. & B. HAMILTON (1927) Bull. Hopkins Hosp. 41, 322.

BOOY, H. L. (1934) *Inleiding tot de physische biochemie.* Haarlem & Leiden.

BOTT, A. A. (1942) *De bepaling van de serumfactor en het quantitatieve erwt onderzoek bij tubereulo-bestrijders tijdens gevoeligheidsbepaling.* Thesis, A'dam.

BROCH, O. J. (1945) Acta med. Scand., Suppl. 166.

BROCH, O. J. (1953) Scand. J. clin. Lab. Invest. 5, 9.

BUNGENBERG DE JONG, H. G. G. and associates (1949) in KRUYT, H. R. (ed). *Colloid Science*, vol. II. Amsterdam.

CARR, CH. W. (1953) Arch. Biochem. 43, 147.

CARR, CH. W. (1953) Arch. Biochem. 46, 417.

CARR, CH. W. (1955) Proc. Soc. exp. Biol. Med. (N.Y.) 89, 546.

CARR, CH. W. (1956) Arch. Biochem. 62, 476.

CHU, H. J. & A. B. HASTINGS (1938) J. clin. Invest. 17, 167.

CONSOLAZIO, W. V. & J. H. TALBOTT (1940) J. biol. Chem. 132, 753.

CONSOLAZIO, W. V. & D. B. DILL (1941) J. biol. Chem. 137, 587.

CORI, C. L. & B. WOLFF (1942) Biochem. J. 36, 413.

CRUYFELD, S. ... AN (1921) Biochem. Z. 123, 304.

CUSHNY, A. R. (1920) J. Physiol. (London) 53, 391.

DANOWSKI, T. S. *et al.* (1955) quoted in ELKINTON, J. R. & T. S. DANOWSKI (eds) *The Body Fluids* (chapter 4). Baltimore.

DANOWSKI, T. S. (1962) Personal communication.

DARROW, D. C. & A. F. HARTMANN (1929) Amer. J. dis. Child. 37, 51.

DARROW, D. C., E. B. HOPPER & M. K. CARY (1932) J. clin. Invest. 11, 701.

DAUTREBANDE, L., H. WHEELER DAVIES & J. MEAKINS (1923) Heart, 10, 133.

DAVSON, H., W. S. DUKE-ELDER & D. M. MAURICE (1949) J. Physiol. (London) 109, 32.

DAVSON, H. (1955) J. Physiol. (London) 129, 111.

DILL, D. B., J. H. TALBOTT & H. T. EDWARDS (1930) J. Physiol. (London) 69, 267.

DILL, D. B., H. T. EDWARDS & W. V. CONSOLAZIO (1937) J. biol. Chem. 118, 635.

DILL, D. B., J. H. TALBOTT & W. V. CONSOLAZIO (1937) J. biol. Chem. 118, 649.

DIXON, M., H. J. & H. W. MOOR (1931) J. Physiol. (London) 73, 149.

DONNAN, F. G. & A. B. HARRIS (1911) J. chem. Soc. 99, 1554.

DONNAN, F. G. (1911) Z. Elektrochem. 17, 572.

DONNAN, F. G. (1924) Chem. Rev. 1, 73.

DOUGHERTY, R. H. & P. JOHNSON (1958) J. phys. Chem. 62, 283.

DOVE, R. F. & P. H. LAVIETES (1942) J. clin. Invest. 21, 781.

DRUCKER, N., A. A. GREEN & A. B. HASTINGS (1939) J. biol. Chem. 131, 641.

we are inclined to see its usefulness *mainly in the fact that from NCE and knowledge* concerning the amounts of complex bound cation and anion an estimate of the net charge of the proteins *in vivo* can be made In normal plasma the difference between NCE and *negative net charge is but small and fairly constant owing both to the fact* that about equal quantities of filtrable anion and filtrable cation are complex bound and to the relatively constant pH value of plasma. But in pathological states the difference *may become far larger because of an increase or decrease in the complex binding* of cations relative to that of anions. In view of the clinical use of hypothermia it will be rather interesting to see whether in dialysis experiments performed at a temperature *of about 25°C the NCE of the plasma proteins will be found 3 mEq/100 g protein* lower as suggested by the experiments of STADIE *et al* (1925) (chapter II), and especially whether the values of the concentration ratios will change in relation to each other

The most intriguing question however is that concerning the NCE *of the non filtrable* ionic substances contained within the cells, and its relation to their net charge. Owing to metabolic processes the changes in pH and in the ionic composition inside the cells *of active organs must be far greater than those which could ever be observed in* plasma *in vivo* Actually the large changes in membrane potential which have been measured for certain living cells might be caused by large changes in the net charge of the non-*filtrable intracellular substances, especially if the pK of their ionogenic* groups is in the range 6 5 to 8 0 As a first orientation towards the possibilities in this respect the determination of NCE and of concentration ratios in equilibrium dialysis experiments with *intracellular or even mitochondrial material* might well prove fruitful Especially the correlation with pH will be of interest.

List of Bibliographical References

ALBERTY R A & H. H. MARVIN (1951) J Amer chem. Soc 73, 3220.

ALDENHOVEN, H. & C. KORTH (1932) Deutsch. Arch. klin. Med. 173, 412.

ANDRES, R., G. CADER & K. L. ZIERLER (1956) J. clin Invest 35 671

ANNINO, J S. & A. S RELMAN (1959) Amer J clin. Path 31 155.

ASTRUP P (1956) Scand. J clin. Lab. Invest. 8, 33.

ATCHLEY D W R F LOEB, E. M. BENEDICT & W M PALMER (1923) Arch. intern. Med 31 611

ATCHLEY D W & E M BENEDICT (1930) J clin. Invest. 9 265

AUSPITZ, H. (1874) Vierteljahrsschrift für Dermatologie und Syphilis 1 275.

BALTZAN, M A R ANDRES, G. CADER & K L ZIERLER (1962) J clin Invest 41 116.

BARKER, S B. & W H. SUMMERSON (1941) J biol. Chem 138 535

BATES, R G (1954) *Electrometric pH Determinations, Theory and Practice* New York.

BENESCH, W M & J SHAFFER (1933) Biochem. Z. 263, 105

BENNHOLD H. (1932) Ergebn inn Med. und Kinderheilkunde 42, 273

BERGMAN, L B ISAKSSON & B. SJÖGREN (1963) Lancet 1 892

BERGSTRÖM, J (1962) Scand J clin Lab. Invest 14 Suppl 68

BERGSTRÖM, J & E. HULTMAN (1962) Lancet 1 1132

BERNSTEIN, R E (1952) S Afr J med Sci 17 101

BIER, A (1906) *Hyperaemie als Heilmittel*, 4th ed Leipzig

BISSELL, G W (1945) Amer J med Sci 210 195

BLACKFAN, K D, & B. HAMILTON (1927) Bull Johns Hopkins Hosp 41 322.

BOOY H L (1936) *Inleiding tot de physische biochemie* Haarlem & Leiden.

BOTS, A A (1942) *De bepaling van de nierfunctie en het quantitatieve urine sediment onderzoek bij tuberculoselijders tijdens goudbehandeling* Thesis, A dam

BROCH, O J (1943) Acta med. Scand. Suppl 146

BROCH, O J (1953) Scand J clin Lab Invest 5 9

BUNGENBERG DE JONG, H G, and associates (1949) in KRUYT H. R (ed), *Colloid Science* vol II Amsterdam

CARR, CH. W (1953a) Arch. Biochem. 43 147

CARR, CH W (1953b) Arch. Biochem. 46 417

CARR, CH W (1955) Proc. Soc. exp. Biol. Med. (N. Y.) 89 544.

CARR, CH. W (1956) Arch. Biochem. 62, 476.

CHU H I. & A. B HASTINGS (1938) J clin. Invest. 17 167

CONSOLAZIO, W V & J H. TALBOTT (1940) J biol. Chem. 132, 753

CONSOLAZIO, W V & D B DILL (1941) J biol. Chem. 137 587

COPE, C. L. & B. WOLFF (1942) Biochem. J 36, 413.

CREVELD, S. VAN (1921) Biochem. Z. 123, 304.

CUSHNY A R. (1920) J Physiol. (London) 53 391

DANOWSKI, T S, *et al* (1955) quoted in ELKINTON, J R & T S DANOWSKI (edd) *The Body Fluids* (chapter 4) Baltimore

DANOWSKI, T S (1962) Personal communication

DARROW D C & A. F HARTMANN (1929) Amer. J dis Child. 37 51

DARROW D C., E. B HOPPER & M. K. CARY (1932) J clin. Invest. 11 701

DAUTREBANDE, L H W DAVIES & J. MEAKINS (1923) Heart, 10, 133.

DAVSON, H W S. DUKE-ELDER & D M MAURICE (1949) J Physiol. (London) 109 32

DAVSON H (1955) J Physiol (London) 129 111.

DILL, D B J H. TALBOTT & H. T EDWARDS (1930) J Physiol. (London) 69 267

DILL, D B H T EDWARDS & W V CONSOLAZIO (1937a) J biol. Chem 118, 635.

DILL, D B J H TALBOTT & W V CONSOLAZIO (1937b) J biol. Chem. 119, 649

DIRKEN, M N J & H. W MOOK (1931) J Physiol. (London) 73, 349

DONNAN, F G & A B HARRIS (1911) J chem. Soc. 99 1554.

DONNAN, F G (1911) Z. Elektrochem. 17 572.

DONNAN, F G (1924) Chem. Rev 1 73

DOREMUS, R H & P JOHNSON (1958) J phys. Chem. 62 203

DOVE, R. F & P H. LAVIETES (1942) J clin Invest 21 781

DRINKER, N A. A GREEN & A B HASTINGS (1939) J biol Chem 131 641

we are inclined to see its usefulness mainly in the fact that from NCE and knowledge concerning the amounts of complex-bound cation and anion an estimate of the net charge of the proteins *in vivo* can be made. In normal plasma the difference between NCE and negative net charge is but small and fairly constant owing both to the fact that about equal quantities of filtrable anion and filtrable cation are complex-bound and to the relatively constant pH value of plasma. But in pathological states the difference may become far larger because of an increase or decrease in the complex-binding of cations relative to that of anions In view of the clinical use of hypothermia it will be rather interesting to see whether in dialysis experiments performed at a temperature of about 25 C the NCE of the plasma proteins will be found 3 mEq/100 g protein lower as suggested by the experiments of STADIE *et al* (1925) (chapter II) and especially whether the values of the concentration ratios will change in relation to each other

The most intriguing question however is that concerning the NCE of the non-filtrable ionic substances contained within the cells, and its relation to their net charge. Owing to metabolic processes the changes in pH and in the ionic composition inside the cells of active organs must be far greater than those which could ever be observed in plasma *in vivo* Actually the large changes in membrane potential which have been measured for certain living cells might be caused by large changes in the net charge of the non filtrable intracellular substances, especially if the pK of their ionogenic groups is in the range 6 5 to 8 0 As a first orientation towards the possibilities in this respect the determination of NCE and of concentration ratios in equilibrium dialysis experiments with intracellular or even mitochondrial material might well prove fruitful Especially the correlation with pH will be of interest.

Klein, A. (1954) C. R. Acad. Sci. (Paris) 239 117
Klotz, J. M. & J. M. Urquhart (1949) J. phys. colloid Chem. 53 100
Kramer, B & F F Tisdall (1922) J. biol. Chem. 53 241

Landis, E. M., L. Jonas, M Angevine & W Erb (1932) J. clin. Invest. 11 717
Landis, E. M. (1934) Physiol. Rev 14 404.
Leeuwen, A. M C. M. Thomasse & P C. Kapteyn (1961) Clin. chim. Acta 6 550
Leeuwen, A. M., an, J J an Daatselaar & L. A. de Vries (1963) Lancet 11 94.
Lewis, G. N & M Randall (1923) *Thermodynamics and the free energy of chemical substances* New York
Liljestrand G (1916) Skand Arch Physiol. 33 153
Lippross, O & W Schoda (1941) M'bach med. Wschr 88 443
Loeb, J (1922) *Proteins and the Theory of Colloidal Behaviour* New York.
Loeb, R F D W Atchley & W W Palmer (1922) J. Gen. Physiol. 4 591
Loeb, R F & E M Benedict (1927) J clin Invest 4 33
Loewy A & N Zuntz (1894) Pflügers Arch ges. Physiol 58 411
Loken, H F R I Havel, G S. Gordan & S L. Whittington (1960) J biol Chem. 235 3654
Love, W D & G E Burch (1953) J Lab clin Med. 41 238

Maizels, C. L H (1946) Yale J Biol Med. 18 419
Maizels, C L H (1947) J biol Chem 169 583.
M nery J F (1954) Physiol Rev 34 334.
Marrack, J (1923) Biochem. J 17 240.
Margaria, R (1931) J Physiol. (London) 73 311
Marriner, T P E E Muirhead, F Jones & J M. Hill (1947) J Lab clin Med 32 1208
McLean, F C & A B Hastings (1935?) J biol Chem 108, 285
McLean, F C & A B Hastings (1935?) Amer J med Sci 189, 601
Miller, J W (1945) J biol Chem 157 173
Miller, J W E F Paccovella & R J Winzler (1949) J biol Chem 177 13
Michaelis, L & P Rona (1908) Biochem Z 14 476
Miller, E C L Head, S Levine, J H Holmes & H Elrick (1961) J Lab clin Med 58, 656
Millikan, G A (1939) Physiol Rev 19 503
Mollin, A F & I J Y Cook (1962) Lancet, I 778
Mollin, A F & I J Y Cook (1962?) Lancet, II 1383
Müller B (1959) Acta med Scand 165 suppl 348
Murphy R R S I Boyle E J ... Jacobson, T M Ba chelor, L T ... & O B M... (1949) Amer J clin Path 19 461
M ... E C T W & E Pomerene (1931) J biol Chem 9 733

Neumann, B S & J B Pincus (1923) J biol. Chem 57 99

Niemiro R. (1955) Bull. Acad. Pol. Sci. Cl. II Vol III No. 5, 167
Northrop J H. & M Kunitz (1925) J Gen. Physiol 7 25.
Northrop J H. & M Kunitz (1926) J Gen. Physiol. 9 351
Northrop J H. & M Kunitz (1928) J Gen. Physiol. 11 481

Osgood, H. C. & J P Peters (1929) J biol. Chem. 81,9
Ottolander, G J A. den (1962) Unpublished results.
Os, G A J & J H. M Koopman-v Eupen (1957) Rec. Trav chim. Pays-Bas 76, 390.
Overman, R. R. & A. K. Davis (1947) J biol. Chem. 168, 641

Pappenheimer, J R. & A. Soto-Rivera (1948) Amer J Physiol. 152, 471
Pappenheimer, J. R. (1953) Physiol. Rev 33 387
Peters, J P H. A. Bulger, A. J Eisenman & C. Lee (1926) J biol. Chem 67 141
Peters, J P A. M Wakeman, A. J Eisenman & C Lee (1929a) J clin. Invest. 6 517
Peters, J P A. M Wakeman, A. J Eisenman & C. Lee (1929b) J clin. Invest 6 577
Peters, J P & D D V Slyke (1932) *Quantitative clinical chemistry* Vol. I, Baltimore.
Peters, J. P & E. B. M (1934) J biol Chem. 107 23
Peters, J P (1935) *Body water The exchange of fluids in man* London
Peters, J P (1952) in Duncan, G. (ed), *Diseases of metabolism*, 3rd ed Philadelphia and London
Peterson, N A G A Feigen & J M Crismon (1961) Amer J. Physiol 201 386
Phillips, R A & F W Putnam (1960) in F W Putnam (ed) *Plasma Proteins*, Vol I (chapter 5) New York
Prasad, A S (1960) Arch intern. Med 105, 560.
Prasad, A S E B Flink & R. Mc. Collister (1961) J Lab clin Med 58 531

Rabinowitz, D & K. Zierler (1962) J. clin. Invest. 41 2191
Recknagel, K. (1930) Z. ges. exp Med. 68, 439
Richter-Quittner, M (1922) Biochem. Z. 126 97
Roborgh L P (1955) *De bepaling van het Na- en K-gehalte van erythrocyten en de betekenis daarvan voor de kliniek* Thesis, Amsterdam
Rona, P & Takahashi (1911) Biochem. Z. 31 336.
Rona, P & H Petow (1923) Biochem Z. 137 356.
Rosenthal, T B. (1948) J. biol. Chem. 173, 25
Rosenthal, S. M (1949) J biol Chem. 179 1235
Roughton, F J W (1935) Physiol. Rev 15 241
Roughton, F J W (1954) in Walter M Boothby (ed) *Handbook of respiratory physiology* Project number 21 2304 0003 of the USAF school of Aviation Medicine
Roughton, F J W (1962) Personal communication.

Sacendi, M A & F W Sunderman (1939) Amer J Physiol 29 17

EDSALL, J T & J WYMAN (1958) *Biophysical Chemistry* vol. I New York.
EICHNA, L. W., A. R. BERGER, B. RADER & W H BECKER (1951) J clin. Invest 30 353.
ELLIOTT H C. & H L. HOLLEY (1951) Fed. Proc. *10*, 180.
EJSMONT W & T RZEPIAK (1959) Bull. Inst. mar med (Gdansk) *10* 115
ENNEKING, J H J (1956) *De quantitatieve e libepaling met behulp van papierelectrophorese en haar toepassing bij een onderzoek over proteinurie* Thesis, Amsterdam.

FANCONI, A & G ALAN ROSE (1958) Quart. J Med. *27* 463
FARBER, S J & M SCHUBERT (1957) J clin. Invest. *36* 1715
FARHI L. E. & H RAHN (1955) J appl. Physiol *7* 472.
FAURHOLT C. (1925) Journal de chimie physique, *2* 1
FAWCETT J K & V WYNN (1956) Brit. med. J *II* 582.
FENN, W O (1928) Amer J Physiol. *85* 207
FERGUSON, J K. W & F J W ROUGHTON (1934) J Physiol (London), *83* 68
FERGUSON J K. W & F J W ROUGHTON (1934) J Physiol (London), *83* 87
FISCH, S., S B. GILSON & R. E. TAYLOR (1950) J ppl. Physiol. *3* 113
FOLK, B D., K. L. ZIERLER & J L. LILIENTHAL (1948) Amer J Physiol. *153* 381
FOSTER, J P (1960) In F W PUTNAM (ed) *The Plasma Proteins*, V l. 1 (chapter 6) New York.

GAMBLE, J L. (1950) *Extracellular fluid* 5th ed., Cambridge, Mass.
GERBRANDY J (1951) *De regulatie van het bloedplasma volume* Thesis, Amsterdam.
GERBRANDY J H B. A. HELLENDOORN, L. A. DE VRIES & A M VAN LEEUWEN (1957) Scand J clin. lab Invest. *10* suppl 31 272.
GERBRANDY J A M VAN LEEUWEN, H B. A HELLENDOORN & L. A. DE VRIES (1960) Clin. Sci *19* 181
GILLIGAN D R., M C. VOLK & H. L. BLUMGART (1934) J clin. I vest. *13* 365
GIUSTINA, G E. MILLA & R. MARGARIA (1953) G Biochem *2* 357
GLUECK, H J L. WAYNE & R. GOLDSMITH (1962) J Lab clin. Med. *59* 40.
GOLLWITZER-MEIER, K. (1925) Z. ges. xp. Med. *46* 15
GOODMAN, DEWITT S. (1958) J Amer chem. Soc. *80* 3892.
GOODM N, DEWITT S. & R. S. GORDON (1958) Amer J clin. Nutr *6* 669
GORDON, R. S. (1955) J clin. Invest. *34* 477
GORDON, R. S. & A. CHERKES (1956) J clin. Invest *35* 206.
GORNALL, A. G CH. J BARDAWILL & M M D VID (1949) J biol. Chem. *177* 751
GORTER, E. & W C. DE GRAAFF (1955) *Klinische diagnostiek* vol. I 7th ed., Leiden.
GREENE, C. H., J L. BOLLMAN, N M KEITH & E. G. WAKEFIELD (1931) J biol. Chem. *91* 203.
GREENE, C. H. & M H. POWER (1931) J biol. Chem. *91* 183
GROLLMAN, A. (1925) J biol. Chem. *64* 141
GROLLMAN, A. (1931) J Gen. Physiol. *14* 661
GURD, F R. N & PH. E. WILCOX (1956) Advances protein Chem. *11* 311
GUTMAN, A. B., E. B. GUTMAN, R. JILLSON & R. D WILLIAMS (1936) J clin. Invest. *15* 475
GUTMAN, A. B & E. B. GUTMAN (1937) J clin. I vest. *16* 903

HALD P M (1933) J biol. Chem. *103* 471
HALD P M & A. J EISENMAN (1937) J biol. Chem. *118* 275
HALD P M (1946) J biol. Chem. *163* 429
HALD P M A. J HEINSEN & J P PETERS (1947) J clin Invest *26* 983
HALPERIN, M H C. K. FRIEDLA D & R. W WILKINS (1948) Amer Heart J *35* 221
Handbook of Respiration (1958) Philadelphia and London.
HARRIS, K E. & H M MARVIN (1927 1929) Heart, *14* 48
HASSELMAN, J J F & E. J VAN KAMPE (1958) Clin. chim. Acta *3* 305
HASTINGS, A. B., D D VAN S YKE, J M NEILL, M. HEIDELBERGER & C. R. HARINGTON (1924) J biol. Chem *60* 89
HASTINGS, A. B. & J SENDROY (1925) J biol Chem. *65* 445
HASTINGS, A. B., H. A. SALVESEN, J SENDROY & D D VAN SLYKE (1927) J Gen. Physiol. *8* 701
HASTINGS, A. B., F C. MCLEAN, L EICHELBERGER, J C HALL & E. D COPE (1934) J biol Chem *107* 351
HENDERSON, L. J (1931) *Le Sang* Paris.
HENDERSON L. J D B DILL, H. T EDWARDS & W O P MORGAN (1931) J biol. Chem *90* 697
HENRIQUES, O M (1928) Biochem. Z. *200* 1
HENRIQUES, O M (1929) Ergebn Physiol *28* 625
HILLER, A J PLAZIN & D D V N SLYKE (1948) J biol. Chem. *176* 1401
HOPKINS, TH., J E. HOW RD & H EISENBERG (1952) Bull. Hopkins Hosp. *91* 1
HOR TH, S. M A RUBIN & E L FOLTZ (1950) Amer J Physiol. *161* 316.
HUCKABEE, W E (1958) J clin. Invest *37* 44
HUCKABEE, W E. (1961) Amer J Med *30*, 833
HUGHES, T R. & J M KLOTZ (1956) in D GLICK (ed) *Methods of Biochemical Analysis* Vol 3 New York

INGRAHAM, R C., C LOMBARD & M B VISSCHER (1932 33) J Gen Physiol *16* 637
I ONE, L. J & D J PE KINS (1962) Biochem. J *84* 152.
JEANNERET P H. ROSENMUND & A. F ESSELIER (1954) Hel med. Act *21* 191

ADDENDUM



SALMINEN S. (1961) *Studies on the ultrafiltrability of serum sodium and potassium.* Thesis, Helsinki.
SALVESEN H. A. & G. C. LINDER (1924) J biol. Chem 58 617
SALVESEN, H A. (1928) Acta med. Scand. 69 1–6.
SANDERSON, P H. (1952) Biochem. J 52, 502.
SCATCHARD G A. C. BATCHELDER & A. BROWN (1944) J clin. Invest. 23 458.
SCATCHARD G A. C. BATCHELDER & A. BROWN (1946) J Amer chem. Soc. 68 2320
SCATCHARD, G (1949) Ann. N Y Acad. Sci. 51 660
SCATCHARD G I H. SCHEINBERG & S. H. ARMSTRONG (1950[I]) J Amer chem. Soc. 72 535
SCATCHARD, G I. H. SCHEINBERG & S. H ARMSTRONG (1950[II]) J Amer chem. Soc. 72 540.
SCATCHARD, G J S. COLEMAN & A. L. SHEN (1957) *J Amer chem. Soc.* 79[I] 12.
SCHMIDT C. (1850) *Charakteristik der Epidemischen Cholera gegenüber verwandten Transsudationsanomalien.* Leipzig and Mitau.
SEEKLES, L. (1936) Arch. Neerl. Physiol. 21 526.
SEVERINGHAUS, J W M STUPFEL & A. F BRADLEY (1956[I]) J appl. Physiol. 9 189
SEVERINGHAUS, J W M. STUPFEL & A. F BRADLEY (1956[II]) J appl. Physiol. 9 197
SHAW L. A. (1927) Amer J Physiol. 79 91
SIGGAARD-ANDERSEN, O K. ENGEL, K. JØRGENSEN & P ASTRUP (1960) Scand. J clin. Lab Invest. 12 172.
SIGGAARD-ANDERSEN, O (1962) Scand. J clin. Lab Invest. 14 587
SIGGAARD-ANDERSEN O (1963) Scand. J clin. Lab. Invest. 15 suppl. 70.
SOFFER, L. J D A. DANTES, E. B. GROSSMAN, H. SOBOTKA & M J JACOBS (1939) J clin. Invest. 18 597
SOFFER, L. J C. COHN E. B. GROSSMAN, M JACOBS & H SOBOTKA (1941) J clin. Invest 20 429
SOULIER, J P & J CROSNIER (1958) Presse Méd 66 617 and 825
STADIE, W C. & E. C. ROSS (1925) J biol Chem. 65 734
STADIE, W C. & H. O'BRIEN (1935–36) J biol. Chem 112 723
STADIE, W C & H O'BRIEN (1937) J biol. Chem. 117 439
STRICKLAND R D M. L. FREEMAN & F T GURULE (1961) Analyt. Chem. 33 545
SUNDERMAN, F W J H AUSTIN & J G CAMAC (1926) J clin. I vest. 3 37
SUNDERMAN, F W (1930) J clin Invest 9 615
SUNDERMAN, F W (1942) J biol. Chem. 143 185
SUNDERMAN, F W (1945) Amer J clin Path. 15 219
SUNDERMAN, F W J F W SUNDERMAN, E. A. F LVO & CH. J KALLICK (1958) Amer J clin. Path. 30, 112.
SWAN, R. C., H. M FEINSTEIN & H. MADDIO (1956) J clin. Invest. 35 607

TANFORD CH. (1950) J Amer chem. Soc. 72 441
TANFORD, CH (1952) J Amer chem. Soc. 74 11
TANFORD, CH., S. A. SWANSON & W S. SHORE (1955) J Amer chem. Soc. 77 6414
TARAIL, R., E. S. HACKER & R. TAYMOR (1952) J clin. Invest. 31 23
TEREPKA, A. R., T Y TORIBARA & P A. DEWEY (1958) J clin. Invest. 37 87
THUNBERG, T (1953) Physiol. Rev 33 1
TORIBARA, T Y A. R. TEREPKA & P A. DEWEY (1957) J clin. Invest. 36 738.
TOBIERON, D C., E. SHEA & R. C. DARLING (1952) J clin. Invest. 31 406.
TSCHIMMER, H & C. TSCHIMMER (1924) C. R Soc. Biol. (Paris) 91 592.

VAHLQUIST B. (1941) *Das Serumeisen. Eine pädiatrisch-klinische und experimentelle Studie* Thesis, Uppsala.
VAN SLYKE, D D A. B. HASTINGS, M HEIDELBERGER & J M NEILL (1922) J biol. Chem. 54 481
VAN SLYKE, D D H WU & F C. MCLEAN (1923) J biol. Chem. 56 765
VAN SLYKE, D D & J M NEILL (1924) J biol Chem. 61 523
VAN SLYKE, D D A. HILLER & K. C BERTHELSEN (1927) J biol. Chem. 74 659
VAN SLYKE, D D A. B HASTINGS, A. HILLER & J SENDROY (1928) J biol. Chem. 79 769
VAN SLYKE, D D & J SENDROY (1928) J biol. Chem. 79 781
VAN SLYKE, D D J R. WEISIGER & K KELLER (1949) J biol. Chem. 179 746.
VAN SLYKE, D D R. A. PHILLIPS, V P DOLE, P B HAMILTON, R. M ARCHIBALD & J PLAZIN (1950) J biol. Chem. 183 349

WALAAS, E. & O WALAAS (1949) Acta physiol Scand. 17 235
WALKER, D G A. S. PRASAD & J SADRICH (1962) J Lab. clin. Med. 59 110.
WALSER, M (1960) J clin. I vest. 39 501
WALSER, M (1961) J clin. I vest 40 723
WALSER, M. (1962) J clin. Invest 41 1454
WAUGH, D F (1954) Advances prot in Chem 11 325
WILLITS, C. O M R. COE & C. L. OGG (1949) J Ass off. gricult. Chem. 32 118.
WILLITS, C. O & C L. OGG (1950) J Ass. off gricult. Chem 33 179
WYMAN, J (1948) Advances protein Chem 4 406

ZIJLSTRA, W. G & C. J MULLER (1957) Clin chim Acta 2, 237
ZIJLSTRA, W G (1958) Personal communication

An *analysis* (of a plasma sample) is understood to consist of a determination of the value x and of the various terms in (1) that contribute to the value y of the sample. The term *experiment* refers to an *in vitro* ultrafiltration experiment, by which a set of samples of various protein contents could be obtained from a single person. Thus, each plasma sample corresponds to a pair of values x_{ij}, y_{ij}, where the subscript i means the number of the experiment, and j the number of the sample within the experiment. Actually we had $1 \leqslant i \leqslant 28$, $1 \leqslant j \leqslant n_i$, where n_i varied from 8 until 13.

2. Selection and arrangement of data. The data which were put at the disposal of the Mathematical Centre for statistical analysis covered 34 experiments; 6 of them fell out since each testee had to be taken into account only once, lest our data become ill-balanced. The remaining 28 experiments could be divided into two groups (denoted by I and II) in a natural way.

The experiments of group I were made in the period from 20-5-60 until 1-12-60, and those of group II from 12-12-60 until 17-11-61; thus, there was some difference of time and of seasons. Moreover, the determinations of Na were performed with a different galvanometer.

It was a lucky circumstance that both groups were of the same order of magnitude (see table), since this made them well suited for comparison.

3. Individual regression lines. For each experiment i (say), Mr VAN LEEUWEN had plotted the values y_{ij} ($j = 1, 2, \ldots, n_i$) against the corresponding values x_{ij}. The diagrams thus obtained could be explained very satisfactorily by admitting, for each testee a linear regression $y_{ij} = \alpha_i x_{ij} + \beta_i$, where the parameters α_i and β_i, referring to a single subject, of course may vary with i.

It was natural, then, to discuss the results on the following simple hypotheses:

(a) The errors of the statistics x_{ij} may be neglected.

(b) The variates y_{ij} ($j = 1, 2, \ldots, n_i$) are independent with expected values $E(y_{ij}) = \alpha_i x_{ij} + \beta_i$ for any i and j.

(c) For any i, j the statistics y_{ij} are normally distributed with a standard deviation $\sigma(y_{ij})$ which is independent of i and j, so we may write $\sigma(y_{ij}) = \sigma$.

(a) was suggested by Prof HOORWEG; his argument was that computations would be much shortened, whereas the loss of information would be small since $\alpha_i\sigma(x_{ij})$ is small as compared to $\sigma(y_{ij})$ (see section 6). Actually he estimated this loss to be less than 10 per cent, which agreed well with the final result. As for (b), the first contention is rather evident, whereas the second was confirmed by the data. Next, by (1), y is a sum of 5 random terms that are presumably independent. Hence, by the Central Limit Theorem, its distribution will be nearly normal. This holds true under very general conditions; in particular it is unnecessary that each component of y should itself be normally distributed. As the number of random terms is increased, the convergence of the sum distribution to a normal one is fairly rapid, provided the standard deviations of the terms be of the same order of magnitude (their expected values are irrelevant in this respect). Now in our case the standard errors of NaCl and HCO_3 did not differ much. As VAN DER WAERDEN (*Mathematische Statistik*, 1957, p. 27) once remarked, 4 is often a big number in Statistics, and here there are always three terms that count (see *l.c.*, p. 247, for the particular case of the approximation to a normal distribution). So only the assumption of a universal standard deviation $\sigma(y_{ij}) = \sigma$ remains open to serious criticism. For the present I pass over possible objections against our statistical model, coming back to it afterwards.

Addendum

1 Concepts and conventions. Th. following conventions are constantly used The symbol $\leqslant$ means *equal or smaller* $b \geqslant a$ is equivalent to $a \leqslant b$ A bar denotes a mean value, *e g*

$$\bar{z} = \sum_{1}^{n} \; i/n$$

is the mean of the sequence $_1$

x is a total protein content, expressed in gm.protein/kg H_2O Omitting symbols [ ] that denote concentrations (as may be done without giving rise to ambiguity) we define

$$y = Na + K + (Ca + Mg) - Cl - HCO_3, \tag{1}$$

where the 5 observed concentrations at the right (in m.aeq /kg.H_2O) are totals including ionized as well as unionized electrolyte

Unlike values that characterize (in a certain respect) an individual (*e g* length or weight) and which appear to be subject to chance if compared with the corresponding values that mark other individuals of the same kind, *population parameters* are constants that depend only on a population as a whole (or rather that depend equally on all individuals belonging to it) and hence characterize it to a certain extent Here they are denoted by Greek letters Examples of parameters are the *population mean* (or *expected value*) the population *standard deviation* and its square usually called the *variance*

True values of parameters are unknown except sometimes when the population is finite These can only be approximately determined by calculating *estimates* from samples of the population Estimates will be denoted by the corresponding Latin letters. Thus, an estimate of the slope α of a regression line is denoted by a in the same way the so-called *standard-error* $s(\;)$ where z may be any estimate is itself an estimate of the standard deviation $\sigma(z)$ of z – The expected value of a random variate is denoted by $E(z)$

In testing a statistical hypothesis by applying it to a particular sample, k means the *tail probability* of the test statistic (T say)

In the cases occurring here, this is the probability that T have a value which (according to the underlying hypothesis) is equal to (or exceeds) the value T calculated for the sample. A small k indicates an improbable sample with respect to our hypothesis, and hence is a reason for rejecting the latter – It is common practice to omit the calculation of k and to decide only whether k is smaller or bigger than certain preassigned values (0 05 or 0 01 say) that are known as *levels of significance* Most statistical tables admit of only a comparison of k with such levels. This, however gives rise to some loss of information, and hence to less gradated final appraisal.

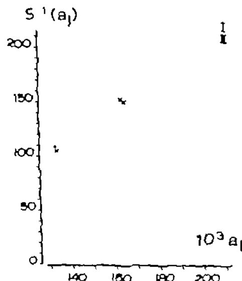
4. Comparison of individual results. The next point concerns the question of whether the calculated regression lines are to be interpreted as particular instances of an ideal regression line, which is valid for all healthy men of medium age, or whether they are (also) noticeably subject to interpersonal variability. The parameters α_i, β_i might well depend, e.g. on minute particularities of the constitution of blood proteins. Such individual variations are to be expected; the question is what we can prove about them.

First, the homogeneity of the variances s_i^2 (or, in other words, the independence of $\sigma(y_{ij})$ of both i and j, as was assumed by (c)) can be tested by Bartlett's test (see e.g. O. KEMPTHORNE, *The design and analysis of experiments* 1952, p. 21). If N independent variances s_i^2 are given, each of which corresponds to a χ^2-distribution with $n_i - 2$ d. of fr. (as should be true for our estimates s_i^2), Bartlett's statistic (a complicated expression, which is here denoted by B) is approximately distributed as χ^2 with $N - 1$ d. of fr. (evidently $N - 1$ has nothing to do with the numbers $n_i - 2$). The approximate character of the test need not bother us: it may be that the test can not be relied upon, but only if $n_i - 2 < 3$ for some values of i; in our case we have $n_i - 2 \geq 6$, and in all but three experiments even $n_i - 2 \geq 8$ (see e.g. J. F. KENNEY and E. S. KEEPING, *Mathematics of statistics* II, 1951, p. 254 seq.).

The result of Bartlett's test was as follows:

Group	N − 1	B	k
I	11	20.6	0.038
II	15	17.2	0.31
I + II	27	40.4	0.047

which indicates (rather significantly) that the data of group I (and, consequently, those of the combined groups) deviate from our assumptions. Presumably the hypothesis of universal ... must be dropped for group I. It remains uncertain in how far the causes of the inhomogeneity of the variances s_i^2 in I have been absent in II, in spite of the value $k = 0.31$ obtained from Bartlett's test.



Next, in the adjacent diagram, the estimated slopes a_i have been plotted against the reciprocals $s^{-1}(a_i)$ of the standard errors. These reciprocals are a natural measure of the 'weight' of an estimate: the bigger its standard error, the less trustworthy the estimate itself. Evidently the small slopes in the diagram have only a medium weight, whereas the big slopes are still worse. The reverse, of course, is not true; yet, on the whole, the medium slopes are of better quality.

Thus the statistics a_i and $s(a_i)$ are, to all appearence, not independent variates. Now it is not clear why this should be so, unless the deviating regression lines are results of experimental errors, too. I see no reason why small fluctuations of a_i, in both directions, should give rise to larger variances $s^2(a_i)$, since the larger variances do not appear to be caused by non-linearity of the regression (which could give a possible explanation). Hence our diagram is evidence for the view that the true slopes α_i are less divergent than is indicated by the values in the table. It remains uncertain to what extent.

Next, the difference of the extreme values a_i in group II is about 3.4 times the maximum of the numbers $s(a_i)$; this could hardly be consistent with a normal distribution of the estimates a_i (and hence, with the hypothesis of practically constant parameters α_i). On the other hand, we had

subject	n_i	s_i^2	$10^3(a_i)$	$10^3 s(a_i)$	$s^{-1}(a_i)$	b_i	$s(b_i)$
group I							
vI e (l)	13	0 161	145	6.5	153		0 59
Gee	13	0 860	168	14.8	67	5 6	1.33
Ott	12	0 159	173	5 7	176	5.8	0.53
dGe (l)	13	0.598	139	10.2	98	11.4	0.6ь
Off	13	0.221	162	6.5	153	7 1	0 63
dVr (l)	13	0 550	182	10.6	94	4.6	1.04
Lie	12	0 436	166	7 6	131	6.6	0.85
vdW	10	0 418	133	9 5	106	8.3	0 98
Jan	11	1.132	201	14.5	69	4.3	1 41
Cej	8	1.092	182	16.1	62	3	1.64
Vro (l)	10	0.410	164	6.6	151	5.2	0 0
vdP	12	0.465	143	10.7	94	7 8	1.02
group II							
vdB	12	0.154	160	4.7	11	8.4	0 48
Rer	12	0 277	169	7 0	142	4.6	0.67
vSo	12	0.321	136	9.0	111	8.6	0.90
Ste	1?	0.300	15	1	141	7 1	0.71
Gou	11	0.433	132	9.1	110	8.0	0.90
Dun	8	0 301	133	8.8	114	7 4	0 85
dRe	11	0.495	184	13.7	73	6.0	1.25
dLo	1	0. 98	184	9 8	102	3 4	0 92
Vre	1	0.538	174	10.	94	6.1	1 12
Bir	1	1.200	180	1 4	57	6 0	1.65
Poo	1	0.543	1 1	11 3	88	4.7	1 11
Lin	12	0.191	1 2	6.9	145	2.8	0 5
Hea	8	0.421	191	13.8	72	4.1	1.3
vOp	12	0.353	143	9.8	102	6.5	1 04
Dou	10	0.287	162	11.8	84	4	1.06
Bos	11	0 677	168	11.3	88	6.2	1 13

The fitting of individual regression lines was done by the method of least squares, which, under the hypotheses (a) (b) (c) gives unbiased estimates of a_i b_i and of their variances $s^2(a_i)$ $s^2(b_i)$ For the results, see the table it follows from the standard errors that the accuracy is somewhat exaggerated (in the computations the accuracy was much more exaggerated in order to avoid rounding-off errors) s_i^2 denotes the estimated variance

$$s_i^2 = \frac{1}{n_i - 2} \sum_{j=1}^{n_i} (y_{ij} - a_i x_{ij} - b_i)^2$$

of the residuals $y_{ij} - a_i x_{ij} - b_i$ (in Mr v L s text NCE = $100\, a_i$ RA = b_i).

For the formulae used see *e g* H CRAMÉR, *Mathematical Methods of Statistics*, 1946, p 549–550 (where the standardization of regression lines is somewhat different)

The calculation of regression lines remains valid even if (c) is false, but (c) is very useful since it provides valuable information concerning the distribution of the estimates and their variances, thus enabling us to check some assumptions underlying our model Thus it follows that a_i and b_i are normally distributed and that $(n_i - 2)s_i^2/\sigma^2$ has a χ^2-distribution with $n_i - 2$ degrees of freedom (abbreviated d of fr)

TABLES BELONGING TO

NET CATION EQUIVALENCY (BASE BINDING POWER) OF THE PLASMA PROTEINS

by

A. M. VAN LEEUWEN

no rea ... ver It is
well k ... sion that
the ar ... xplained
in twc ... as, or by
assum ...
Fin ... applying
analy ... for group
II Th ... he 0.1 per
cent p ... regression
line, I ... basic as
samp ...

... oduction
... bout the
... neral be

5. Weighe ...
of weights ...
weight of ...
somewhat ...

... ement of
... , but still
where $\bar{a}$ ... nt of the
the value ... e for the
quite sati ...
weighed ...
correctne ... bution of
... We had
6. Additi ... those of
the varia ... d in the
the defin ...
group I ... lying the
meantim ...
Mr V ...
formula ...

... u_i u_i are

where λ ... errors From any pair of corresponding observations The asterisk refers to accidental errors From his data $s^{*2}(x) = 0.1331$ $s^{*}(y) = 0.0873$ By assuming the values x_{ij} to be exact we add $\alpha^2\sigma^2(x)$ to the variance $\sigma^2(y)$. Of this additional term we have an estimate $\alpha^2 s^{*}(x) = 0.0034$ Hence Prof HEMELRIJK s argument.

C. G G VAN HERK

Mathematical Centre, Amsterdam

TABLES BELONGING TO

NET CATION EQUIVALENCY (BASE BINDING POWER) OF THE PLASMA PROTEINS

by

A. M. VAN LEEUWEN

substrate	method	n*	pH range	NCE at pH 8	NCE increase per unit pH increase	authors
				mEq per 100 g prot		
			7 7.7	17.3	8.8	VanSlyke et al. (1923)
				20.3	9.7	Hastings et al (1927)
			6.5 7.8	27.3 18	12. 7.7	VanSlyke et al. (1928)
			6 8.0	22.3	10	
			tem D (chapter II)	23.6	10	
			7.3 7.6	27.2 7	1	Darrow Hartmann (1923)
			6 8.5		10.9	Henderson et al (193)

n* number of samples studied

ERRATA

tabl 2 — For explanation asterisks see page 42 in text.

tabl 5 6 7 32 — CO_2 not in mEq/L but in mM/L.

table 37 — protein concentrations in g/Kg H_2O
number of samples from which correlation between w te content and protein concen tration was calculated.

EXPLANATION

The tables are indicated by two numbers the first is en circled and represents the actual number of the table the second number refers to the page in the text on which the table is first mentioned For example

(13) 69 *means table 13 first referred to on page 69*

Author(s)	Year	Na method	Total base method
Overman Davis	[illegible]	(3a)	
Walser Walser	[illegible]		(A)
Walser Walser	[illegible]		(B)
Elliot McCrory	[illegible]	(3a)	
Tarail et al.	[illegible]	(3a)	
Bernstein	[illegible]	(3a)	
Peters	[illegible]		
Love Burch	[illegible]	(3a)	
Jeanneret et al.	[illegible]	(3a)	
Danowski et al.	[illegible]	(3a)	
Bergholt	[illegible]	(3a)	
Bergholt	[illegible]	(3a)	
[illegible] Wynn	[illegible]	(3a)	
Fawcett Wynn	[illegible]	(3a)	
[illegible] Sanderson	[illegible]	(3b)	
Amdisen [illegible]	[illegible]	(3a)	
[illegible]	[illegible]	(3a)	
[illegible]	[illegible]	(3a)	
[illegible] Cook	[illegible]	(3a)	
Bergström [illegible]	[illegible]	(3a)	

Notes Methods used for Na determination
(1) gravimetric analysis
(3a) flame photometry, plasma direct
(3b) flame photometry, deproteinized plasma
(4) neutron activation analysis

Methods used for total base determination
(A) method of Fiske or modification
(B) electrodialysis

When the Mg concentration is placed between brackets the value was assumed by us. The corresponding values for 'rest cations' and RCE are then also placed between brackets.
Fawcett, Wynn and Amdisen [illegible] reported values for total CO_2; the HCO_3 concentration was calculated by us assuming a pH value of [illegible].

S(erum) or P(lasma)	[illegible]	F(emale) or M(ale)	number of subjects	protein g/L	CATIONS Na	K	Ca	Mg	sum cations	total base	ANIONS Cl	HCO_3	inorg. ph.	Cl + HCO_3	rest Cl HCO_3	HCE	METHODS Na	total base	REFERENCES		
					m Eq p l l																
P					148	5	5	3	35		103	27	3	130	25	19			Gamble	[illegible]	
S			7		146.0	5.0	5.0	2.5	155.5								(1)		Krane & Tisdall	1922	
P					136.5						102.8	27.7	1.7	130.3			(1)		Marrack	1933	
S	f		7		145.8	5.1	5.0	1.6	156.0								(1)		Salvesen & Linde	1934	
S			9							164.7	104.0							(A)	Sunderman et al.	1929	
P	f		10	66						155.7	103.8	28.6	2.3	129.3	25.4	30.4		(A)	Peter et al.	1928	
S		M	9	68	147.6	5.4	5.4	2.2	80.7		1 4 8	28.0	2.1	132.8	27.9	21.9	(1)		Salvesen	1928	
S		F	6	67	142.1	4.8	5.2	2	154.6		100.2	28.4	2.4	133.6	21.0	16.0	(1)		Salvesen	1928	
S			10							153.8								(A)	Gard & Peter	1929	
S			13	71						150.7	100.0	24.7	2.2	124.7	29.0	20.0		(A)	Darrow & Hartmann	1929	
S			10	76						151.9	103.5	29.0	1.0	133.5	19.4	13.4		(A)	Atchley & Benedict	1930	
S			10	72	78.0	4.0	6.2	3.0	144.2	6.3	102.(						(1)	(A)	Sunderman	1930	
S	f		8	68	139.2	3.3	4.7	(3.0)	(1.0 [illegible])		106.1	24.6	2.2	130.7	(18.6)	(13.5)	(1)		Dill et al.	1930	
S	p		10	71	134.6	4.6	5.0	7	45.6	6.5	103.2	27.0	2.3	30.3	10.1	10.1	(1)	(A)	Hald	1933	**
S	p		10		135.1	4.6	6.3	1.6	44.0	1 5 8							(1)	(A)	Hald & Eisenman	1937	
S			13	73	140	5.3	5.6	(3.0)	(33)		104.8	25.1		130.7	(22.6)	(16.0)	(1)		Dill et al	1937[1]	
S	p		37		140.2		5.0	2.7	153.5	53.1							(1)	(B)	Consolazio & Talbott	1940	
S	p		7		142.8	3.8	6.1	(2.6)	(63.4)	154.1							(1)	(B)	Consolazio & Talbott	1940	**
S		M	10							154.3								(B)	Brock	1943	
S		F	10							154.3								(B)	Brock	1943	
S			8		137.1												(1)		Hald		**

Method	Authors	Year
[illegible]	[illegible] et al.	[illegible]
[illegible]	[illegible] et al.	[illegible]
(IIa)	[illegible] et al.	[illegible]
(IIb)	Overman Davis	[illegible]
(B)	[illegible]	[illegible]
(B)	[illegible]	[illegible]
(IIa)	Elliot Bailey	[illegible]
(IIa)	[illegible] et al.	[illegible]
(IIa)	[illegible]	[illegible]
	[illegible]	[illegible]
(IIa)	[illegible]	[illegible]
(IIa)	[illegible] et al.	[illegible]
(IIa)	[illegible] et al.	[illegible]
(IIa)	[illegible]	[illegible]
(IIa)	[illegible]	[illegible]
(IIa)	[illegible] Wynn	[illegible]
(IIa)	[illegible] Wynn	[illegible]
(IIb)	[illegible]	[illegible]
(IIa)	[illegible]	[illegible]
(IIa)	[illegible]	[illegible]
(IIa)	[illegible]	[illegible]
(IIa)	[illegible] Cook	[illegible]
(IIa)	[illegible]	[illegible]

Notes: Methods used for Na determination
(1) gravimetric analysis
(IIa) flame photometer, plasma direct
(IIb) flame photometer, deproteinized plasma
(3) neutron activation analysis

Methods used for total base determination
(A) method of Fiske or modification
(B) electrodialysis

When the Mg concentration is placed between brackets the value was assumed by us; the corresponding values for 'base cations' and NCE are then also placed between brackets.
[illegible] Wynn and [illegible] [illegible] reported values for total CO_2; the HCO_3 concentration was calculated by us assuming a pH value of [illegible].

substanc	concentration mEq pe litre plasma	ref renc	furthe remarks
SO_4	0 7	0 7 mEq/L Loeb & Benedict (1927)	
		0 65 mEq/L Mill t al (1961)	
lactat	0 8	0 78 mEq/L Andre et al (1956)	
		0 64 mEq/L Huckabee (1956)	aft 1 hr complete rest
		1 04 mEq/L Huckabee (1961)	recooval scent (in bed)
non-esterified fatty acids	0 6	Gordon & Cherkes (1956)	
citrate	0 1	Thunberg (1953)	
keto acid	0 2	Rosenthal (1949)	

The values given fo lactate refer to blood

DETAILS OF TECHNIQUE

In vitro experiments

[illegible]

In vivo observations

[illegible]

Notes: Methods used for the determination of [illegible] (a) [illegible] (b) [illegible] (c) [illegible]

[illegible]

substanc	oncentration mEq pe litre plasma	references	furthe rema k
SO_4	0 7	0 7 mEq/L Loeb & Benedict (1927) 0 65 mEq/L Mill t al (1961)	
lactat	0 8	0 78 mEq/L Andre et al (1956) 0 64 mEq/L Huckabee (1958) 1 04 mEq/L Huckabee (1961)	ft 1 hr omplet rest reconval scent (in bed)
non-esterified fatty acid	0 6	Gordon & Cherkes (1956)	
citrate	0 3	Thunberg (1953)	
keto acid	0 2	Rosenthal (1949)	

The value give fo lactat refer to blood

	prot g/L		Na		K		Ca+Mg		Cl		CO_2	
					mEq/l							
temp. de la centrifug (°C)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
in vivo ultrafiltration I	72,0	72,0	138,5	138,5	4,1	4,04	5,81	5,70	106,4	106,6	24,9	24,3
	62,7	63,7	138,0	138,0	4,03	4,06	6,04	6,04	104,3	104,0	20,0	23,9
	62,0	61,7	138,9	138,0	4,10	4,16	6,43	6,04	101,0	[illegible]	24	[illegible]
	62,9	64	136,5	138,5	4,35	4,31	7,00	[illegible]	10 4	101,5	[illegible]	23,1
in vivo ultrafiltration II									101,3	103,2	24	23,5
									102	102,6	24,7	23,7
									10 5	10 8	23,6	23,3
									96	100,5	23,5	24

(5) 62

	small tube		large tube	
	Cl	CO_2	Cl	CO_2
	mEq/L		mEq/L	
fou consecutive sampl from an in vivo ltrafiltration experiment	98.9	27.6	98.7	27.8
	97.9	28.9	97.8	28.7
	97.4	28.3	97.6	28.3
	97.4	29.2	97.3	29.3

(6) 62

	CO_2	
	tube	syringe
ampl	mEq/L	
1	29.9	30.2
2	30.2	30.2
3	28.8	29.6
4	29.6	29.4
5	28.5	28.6
6	28.2	28.3
7	28.8	27.0
8	28.6	28.4

	Kjeldahl	biuret	Kjeldahl minus biuret
plasma from normal subjects (in vivo ultrafiltration experiments)			
vL	73.3	73.	0.0
	62.7	62.5	0.2
	93.6	94.	0.3
V	110.7	1 [illegible]	0.2
Pe	62.2	61.0	1.0
	87.6	82.2	2.2
	108.	108.	-0.1
pooled plasma from patients (most abnormal serum proteins or proteins)			
	0.7	68.	0.1
3	[illegible]	70.1	0.2
3	64.1	63.	0.
	70.9	70.3	0.
	64.6	3	0.2
6	77	77.0	-0.3
	75.5	75.3	0.2
human serum albumin			
	70.1	70.	0.3
plasma from patient			
Mo (blood re 300 mg%)	[illegible]	57	-0.7
vO (myeloma)	37.1	37.0	0.1
Ma (myeloma)	92.	93	2
Pe (myeloma)	98.	96.7	
	94.	97.7	3.0
	65.3	64.	3.6

Note: indicates plasma samples diluted with saline solution

	[illegible]						
pairs of duplicate determinations	27	13		05	18	03	61
concentration range (g/L)	54 - 64	64 - 72	72 - 83	83 - 93	93 - 104	104 - 114	114 - 130
SD of single determination in plasma (g/L)	0.34	0.38	0.43	0.35	0.37	0.31	0.34
SE of mean of duplicate determination (g/L)	0.25	0.29	0.33	0.25	0.19	0.20	0.25

(8) 63

m ip l tl n	p H b l d	
	exp. 1	exp 2
6 ml blood drawn anaerobically int syringe	7,240	7,306
afte adding 2 ml ai gentl mixing and expulsion of air	7,244	7,304
fte adding anothe 2 ml ai repeatedl pulling out plunge t the 12 ml mark with syringe capped, and expulsion of air	7,250	7,305

(9) 63

	p H b l d			% HbO_2 blood	
	exp 1	xp. 2	exp 3	exp. 4	exp. 5
directly from syringe	7.342	7,362	7.325	39	41
after transfe to testtube	7,344	7,355	7,310	36	38

Note testtube filled beforehand with alveolar air and closed with rubber cap

(10) 64

	w t e t t		
triplet of triplicat det rminations	67	60	56
range of wat content (g/L)	930 930	930 910	910 940
SD of singl det rmination in plasma (g/L)	0,41	0,43	0,44
SE f th mean f triplicat determination (g/L)	0,24	0,26	0,25

samples	[illegible]			plasma minus [illegible] plasma		deproteinized plasma minus plasma	
	prot g/L	Na mEq/L	K	Na mEq/L	K	Na mEq/L	K
1	[illegible]	5	4.50	2.0	2.0	3.0	0.21
		30.5	[illegible]		1	2.6	0.00
3	[illegible]	138.3	[illegible]		[illegible]	1.0	0.2
		2.5		-0.3	[illegible]	1.0	0.0
		137.6	4.15	1	-0.02	2.0	0
		137	4	0	-0.7	-0.2	[illegible]
6		1	3.5	-0.0	0.33	1	[illegible]
7		137	2.00		-0.04		0.3
8		138	3.31		-0.03		0.11
9		1.9		0.5			
1		39	50	0	[illegible]		-0.20
2		46	2.76	0	[illegible]	2.3	0.44
3		140.9		0			
		32		0.2			
5		37.0		-0.3			
1		38		0			
			25	-0.2	0.1		
[illegible]	4.3	37		0.2	0.07		
	64	36	40		-0.30		
	1	36	4.30	-0	0.10		
[illegible]	64	32.5	40		-0.39		
	5		79		-0.2		
[illegible]	36.8	128	[illegible]	0.1	-0.31		
	64	22	25	-0.3	-0.31		
[illegible]	64	15	52		-0.32		
	4	1	40	2	-0		
[illegible]	42	343	76	-0	-0.14		
	62	22		-0	0		
[illegible]	64	46	4.25		-0.07		
	66	48.0	1	0.0	[illegible]		
	66	48.0	4.0		[illegible]		
		48.0		0.0	[illegible]		
[illegible]	76	14	90	0	02		
	6.0		4.2	-0	05		
	6	33.6	5.00		-0.0		
			[illegible]	-0	[illegible]		
[illegible]	61	136	3.90		-0.30		
	61	36	3.90		-0		
	64	34	4		-0.04		
	64	36			-0.04		
[illegible]	63.4	39	2	-0	-0		
	69	39	2.50	1	-0.32		
[illegible]		36		-0	0		
	61	3	46	0	0.03		
[illegible]		36	32	0.6	2		
	64	37	33	0	-0		
		mean value		-0.10	0.[illegible]		
		maximum value		0	2.00		
		minimum value		1	0.3		

Note [illegible] the sampl [illegible] from patients with [illegible] when these also are left out we calculate from the samples with known protein content the following mean values

protein content of samples	67.0 g/L
Na (plasma minus [illegible] plasma)	-0.11 mEq/L
K (plasma minus [illegible] plasma)	-0.100 mEq/L

(13) 69

	plasma	fraction I	plasma fraction I
albumin	37,3	0,0	37,3
α_1 α_2 and β-globulin	18,4	1,8	32,0
fibrinogen	2,8	11,2	14,0
γ globulin	18,2	6,3	12,5

all values are in g/L

(14) 72

Difference between N and K values found with 'deproteinized plasma' and those found with 'plasma'

number of samples		67	61	18
protein concentration	mean g/L	68,8	91,1	110,0
	range	60 to 80	80 to 100	100 to 125
N ('deproteinized plasma minus 'plasma')	mean mEq/L	2,1	2,6	2,8
	range	0,6 to 3,9	0,8 to 3,8	1,1 to 4,8
K ('deproteinized plasma minus 'plasma')	mean mEq/L	0,13	0,10	0,14
	range	-0,13 to 0,38	-0,13 to 0,31	0,03 to 0,40

samples	plasma prot g/L	plasma Na mEq/L	plasma K mEq/L	'plasma minus ashed plasma' Na mE/L	'plasma minus ashed plasma' K mE/L	'deproteinized plasma minus 'plasma' Na mEq/L	'deproteinized plasma minus 'plasma' K mEq/L
					1	1	0.21
				0.			
1	not determined but below 100	135.6	99			1.	00
				0 0	-0 1		
2		136.5	96			0	2
					00		
3		13.3	3 96				09
				-0 3			
		2 5				2	-0 1
					-0 2		
5		137	4.			-0 2	0
					-0 7		
6		137 0	7			1 0	0.30
		13.1	3 16	-0.	-0.32	1 0	10
				0	-0.00		
		137 4	3.90			1 0	1
				0.	-0 04		
9		139 5					
1		1.5		0.6			
		13.1	4.00		0.00	4	-0.20
		140.	2.70		0 00	2	0.60
2				2			
		40.9					
3				0.			
14		133.					
		0		-0			
				1			
6		138.4					
1		132	4.20	-0.2	-0.10		
		137	00	0.2	-0.07		
Bo	4.9						
	104.	30.3	40	0.0	-0.30		
	72.	31.	4.30	-0.	-0.10		
			60	1	0.30		
K	04.						
		3 9	4.79	0.1	-0.		
Fr*	136.	123.	00	0.1	-0 1		
	64 2	72.	29	-0.3	-0 2		
					0.32		
Mo	94.	73 1					
		1	40	0.2	-0.		
		41	76	-0.	-0.		
	1	23.	4.	-0.	-0. 3		
			23		-0.07		
Bi	60.						
	60.	40.	4.1		00		
	00 0	40.	4.		00		
	2 1	40.	0	0.0	-0.04		
W	70.	34	40	0.	02		
		34.	92	-0.	00		
	07	33.	3.00	-0.	-0		
	94 3	73	03	-0.	00		
Ge	03.	34.	40	0.	-0.30		
	03.	30.	3 96		-0.		
	60.	38.	4.		-0 00		
		34	0	-0	04		
On	52	73		-0.	-0. 0		
		73	2 50		-0.		
Th		36		0.	0 0		
	07.	37		1	00		
De	73.		72		-0.12		
	60.	37	23	0.2	-0		
		mean value		0.10	-0. 00		
		maximum value			00		
		minimum value			-0.32		

Note indicates the samples from patients with myeloma when these values are left out calculate from the samples with known protein content the following mean values

protein content of samples	87.0 g/L
Na ('plasma minus 'ashed plasma')	-0.11 mEq/L
K ('plasma minus 'ashed plasma')	-0. 00 mEq/L

(16) 73

plasma sampl	apparent prot in conc g/L	Na measured mins expected (mEq/L)	K measured mins expected (mEq/L)
I	36	0.2	0.02
(Na content 1.2 mEq/100 g prot)	72	0.3	0.04
(K content 0.08 mEq/100 g prot)	144	-0.8	-0.12
	216	1.2	-0.16
II	63	0.1	
(N ontent 1.1 mEq/100 g prot)	189	-0.5	
III	36	0.0	
(N content 0.0 mEq/100 g prot)	72	0.1	
	108	-0.3	
	144	-0.8	
	180	1.3	
IV	30	0.0	
(N content 1.1 mEq/100 g prot)	60	0.3	
	90	0.4	
	120	0.6	
V	180	0.0	
(N content 0.0 mEq/100 g prot)	180	0.0	
	180	0.0	

Note th value given for plasma V represent the results of three separat xperiments The apparent protein concentration ref rs t th protein oncentration in the solutions analysed but is expressed the protei oncentration f hypothetical plasma which in 1 100 dilution would have the sam protein concentration as the solution actually analysed

(17) 75

consecutive determinations on one sampl f plasma	m h d A: Na (mEq p l l)	m h d A: K	m th d B: Na	m th d B: K
1	138.3	4.93	138.6	5.18
2	138.6	4.97	138.6	4.90
3	138.7	5.00	137.9	4.85
4	138.4	5.00	140.4	5.27
5	138.4	5.00	138.0	4.95
6	138.3	5.01	140.0	5.00
7	138.7	5.18	138.8	5.03
8	138.5	4.97	138.3	5.05
9	138.6	4.97	138.3	5.13
10	138.7	5.01	138.9	5.03
mean valu	138.52	5.004	138.28	5.040
maximum value	138.7	5.18	140.4	5.27
minimum value	138.3	4.93	138.6	4.83

(18) 75

	Na	K
[illegible] of duplicate determinations	[illegible]	[illegible]
concentration range (mEq/L)	[illegible]	[illegible]
SD of single determination in plasma (mEq/L)	[illegible]	0.011
SD of the mean of duplicate determination (mEq/L)	[illegible]	[illegible]

(19) 77

	Ca	[illegible]
pairs of duplicate determinations	[illegible]	[illegible]
concentration range (mEq/L)	[illegible]	[illegible]
SD of single determination in plasma (mEq/L)	[illegible]	[illegible]
SD of the mean of duplicate determination (mEq/L)	[illegible]	[illegible]

[illegible] the SD of the mean of duplicate determination calculated at [illegible]

(20) 78

serum dialysed against dist. water	NaCl standard solution ml	serum ml	[illegible] ml	Cl [illegible] mEq/L	Cl corrected for Cl in serum mEq/L
	[illegible]		[illegible]	[illegible]	[illegible]
I		[illegible]		[illegible]	[illegible]
[illegible]		[illegible]	[illegible]	[illegible]	[illegible]
[illegible] g/L	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]		[illegible]	[illegible]	[illegible]
II		[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible] g/L	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

(21) 78

[illegible]	Cl plasma	Cl deproteinized plasma
	m[illegible]/L	
I	[illegible]	[illegible]
II	[illegible]	[illegible]
III	[illegible]	[illegible]
IV	[illegible]	[illegible]

[illegible]

(16) 73

plasma samples	apparent prot in conc g/L	Na measured minus expected	K measured minus expected
		mEq/L	
I	36	-0.2	0.02
(N content 1.2 mEq/100 g prot)	72	-0.3	0.04
(K content 0.05 mEq/100 g prot)	144	0.6	0.12
	216	1.2	-0.16
II	63	0.1	
(N content 1.1 mEq/100 g prot)	189	0.5	
III	36	0.0	
(N content 0.0 mEq/100 g prot)	72	0.1	
	108	0.3	
	144	-0.6	
	180	1.3	
IV	30	0.0	
(N content 1.1 mEq/100 g prot)	60	0.3	
	90	0.4	
	120	0.6	
V	180	0.0	
(Na content 0.0 mEq/100 g prot)	180	0.0	
	180	0.0	

Note the values given for plasma V represent the sults of three eparat experiments Th apparent protein concentration refers t the protei concentration in the solutions analysed but I expressed as the prot in oncentration of ypothetical plasma which in 1 100 dilution would have the sam protein concentration as the solution actually analysed

(17) 75

consecutive determinations on one sampl of plasma	m th d A		m th d B	
	N	K	Na	K
	m E q p		l i t	
1	139.3	4.93	138.6	5.10
2	138.6	4.97	138.6	4.90
3	138.7	5.00	137.9	4.85
4	139.4	5.00	140.4	5.27
5	138.4	5.00	139.0	4.85
6	138.3	5.01	140.0	5.00
7	139.7	5.10	138.8	5.03
8	138.8	4.97	138.2	5.05
9	139.6	4.97	138.3	5.12
10	139.7	5.01	138.9	5.05
mean valu	138.97	5.004	139.28	5.040
maximum value	139.7	5.10	140.4	5.27
minimum value	138.3	4.93	136.8	4.85

(18) 75

	Na	K
pairs of duplicate determinations	143	143
concentration range (mEq/L)	130 144	2.4 [illegible]
SD of single determination in plasma (mEq/L)	0. [illegible]	0.011
SE of the mean of duplicate determination (mEq/L)	[illegible]	0.008

(19) 77

	C	Ca+Mg
pairs of duplicate determinations	105	104
concentration range (mEq/L)	3 [illegible]	4.5 [illegible]
SD of single determination in plasma (mEq/L)	0.017	0.017
SE of the mean of duplicate determination (mEq/L)	0. 12	0.0

or Mg the SE of the mean of duplicate determination was calculated at 0.017

(20) 78

serum dialysed against dist. water	NaCl standard solution	serum	diluted H_2SO_4 in gelatin	Cl measured	Cl corrected for Cl in serum
	ml	ml	ml	mEq/L	
I	5			100.	[illegible]
(protein		0.5	7	[illegible]	[illegible]
43 g/L)	0.5	0.5	7	[illegible]	100.1
	[illegible]	1.0		[illegible]	[illegible]
	5	2	7	1 [illegible]	100.7
II	5			[illegible]	[illegible]
(protein		1.0	7	[illegible]	[illegible]
36 g/L)	[illegible]	1.0	7	[illegible]	100.
	0.5	2.0	7	1 1.0	[illegible]

(21) 78

Cl

samples	plasma	deproteinized plasma
	mEq/L	
I	[illegible]	[illegible]
II	[illegible]	[illegible]
III	101.9	100
IV	101.9	[illegible]

Note concerning experimental procedure

plasma [illegible] 5 ml plasma [illegible] ml [illegible] after centrifugation [illegible] ml added to [illegible] ml ([illegible]) [illegible] % gelatin) titration with $AgNO_3$

deprot plasma [illegible] 5 ml plasma 3 ml [illegible] ml [illegible] % trichloroacetic acid after centrifugation [illegible] supernatant added to [illegible] ml ([illegible]) [illegible] % gelatin) [illegible] ration with $AgNO_3$

standard solutions treated in the same manner. slope of titration-curve and point of equivalence not altered in the presence of trichloroacetic acid reagent blank the same for plasma and for deprot plasma

(16) 73

plasma sample	apparent protein conc g/L	Na measured minus expected mEq/L	K measured minus expected mEq/L
I	36	0.2	-0.02
(N content 1.2 mEq/100 g prot)	72	0.3	0.04
(K content 0.06 mEq/100 g prot)	144	0.6	0.12
	216	1.3	0.16
II	63	0.1	
(N content 1.1 mEq/100 g prot)	189	-0.6	
III	38	0.0	
(N content 0.9 mEq/100 g prot)	72	0.1	
	108	-0.2	
	144	-0.8	
	180	1.3	
IV	30	0.0	
(Na content 1.1 mEq/100 g prot)	60	-0.3	
	90	0.4	
	120	0.6	
V	180	0.0	
(N content 0.0 mEq/100 g prot)	180	0.0	
	180	0.9	

Note the value given for plasma V represent the result of three separat experiments The apparent prot in concentr tion ref s to the prot i concentration in the solutions analysed but i expressed as the protei concentration f hypothetical pl ma which in 1 100 dilution would have the same prot in concentration the solution actually analysed

(17) 75

consecutive determinations on one sample of plasma	m th d A Na	K	m th d B N	K
	m E q	p	1 1 l	
1	138.3	4.93	138.6	5.18
2	138.8	4.97	138.6	4.90
3	139.7	5.08	137.9	4.85
4	138.4	5.00	140.4	5.27
5	138.4	5.00	138.8	4.85
6	138.3	5.01	140.0	5.00
7	138.7	5.10	138.8	5.03
8	138.8	4.97	138.3	5.05
9	138.6	4.97	138.3	5.12
10	138.7	5.01	138.9	5.05
mean value	138.82	5.004	138.76	5.040
maximum value	139.7	5.10	140.4	5.27
minimum value	138.3	4.93	138.6	4.85

(24) 100

		HbO_2 %	P_{O_2} mm Hg	pH	P_{CO_2} mm Hg
precompression	mean	63	34	7.352	46.3
	range	(28 68)		(7.31 7.40)	(39 54)
endocompression*	mean	10	15	7.294	54.4
	range	(7 23)		(7.26 7.33)	(48 62)
postcompression	mean	56	31	7.347	48.8
	range	(30 81)		(7.32 7.38)	(39 57)

Note endocompression refers to values obtained from 25 to 40 minutes after the start of compression.

(25) 102

		number of observ	pH decrease during compression: mean	range
van LEEUWEN et al (196)	normal subjects and patient	63	-0.05	-0.02 to -0.09
present study	normal subjects	34	-0.04	0.00 to -0.10
	patients	28	-0.05	-0.02 to -0.13

Note pH decrease of more than 0.09 was only observed in three instances twice it wa 0.1 once 0.13

(26) 103

	lactate (venous blood) mEq/Kg H_2O: mean	range
precompression	1.50	0.83 to 2.10
compression (5 to 20 min)	1.5	1.00 to 2
compression (25 to 40 min)	1.50	1.04 to 2.03
postcompression	1.33	0.77 to 2.18

(27) 110

Precompression value minus postcompression value

protein g/Kg H_2O	pH	Na	K	cations	Cl HCO_3	lactate	(cations minus Cl+HCO_3+lactat)
		m E q / K g H $_2$ O					
0	0.01	0	-0.40	-0.40	-0.08	0.23	0.25

(22) 78

containe	mpl hours fter start dialy i	N flam mEq/L	Cl potent tit mEq/L	N minus Cl	Cl Valhard mEq/L	Na minus Cl
I	0	153.2	153.2	0.0	153.3	-0.1
	16	152.7	152.8	-0.1	151.4	1.7
	24	153.7	153.6	0.1	152.5	1.2
	40	153.6	154.4	-0.8	153.3	+0.3
II	0	153.2	153.2	0.0	152.7	+0.5
	16	153.2	153.2	0.0	152.9	0.3
	24	152.9	153.2	-0.3	152.1	+0.8
	40	153.1	153.4	-0.3	152.7	+0.4
III	0	154.7	154.6	0.1	154.2	0.5
	16	154.7	154.4	+0.3	154.6	0.1
	24	155.1	155.0	0.1	153.8	1.3
	40	154.6	155.0	0.4	153.2	1.4
IV	0	152.6	152.4	+0.2	150.5	2.1
	16	152.5	152.6	-0.1	150.9	1.6
differenc between N and Cl conc			m ange +0.3 t	0.13 -0.8	2.1 t	+0.57 1.7

(23) 98

subject and sample		prot in g/Kg H_2O	alb	α_1 gl	α_2-gl	β-gl	γ gl
			percentage of total prot in				
Do	1	72.7	58.7	3.7	8.1	7.2	21.3
	2	79.2	58.2	3.1	8.5	6.8	21.4
	3	82.1	58.3	3.5	8.4	6.9	21.0
	4	87.7	58.8	3.3	8.6	7.2	21.2
	5	112.2	58.0	2.5	10.0	7.0	21.6
	6	113.2	58.0	4.2	10.0	6.7	20.1
	7	88.1	58.6	3.4	8.9	7.3	21.7
	8	77.1	58.1	3.9	8.2	6.8	21.1
	9	73.7	58.2	4.0	8.9	6.8	21.1
B	1	67.0	62.2	3.4	7.1	7.2	20.1
	2	67.8	60.4	3.8	7.1	7.8	21.0
	3	80.1	60.4	3.7	7.0	7.6	21.3
	4	93.7	60.7	3.5	7.8	7.3	20.8
	5	108.2	58.8	3.7	7.9	8.4	20.1

(24) 100

		HbO_2 %	P_{O_2} mm Hg	pH	P_{CO_2} mm Hg
precompression	mean	[illegible]	30	7.383	40.3
	range	(20 [illegible])		(7.31 7.40)	([illegible] [illegible])
endocompression*	mean	10	15	7.204	64.4
	range	(7 33)		(7.20 7.32)	([illegible] 82)
postcompression	mean	[illegible]	31	7.347	48.8
	range	([illegible] [illegible])		(7.32 7.38)	(35 [illegible])

Note endocompression refers to values obtained from 25 to 40 minutes after the start of compression.

(25) 102

		number of observ.	pH decrease during compression mean	range
van LEEUWEN et al (1961)	normal subjects and patients	[illegible]	-0.06	[illegible] to -0.08
present study	normal subjects	34	-0.04	0.00 to -0.10
	patients	[illegible]	-0.05	-0.02 to -0.13

Note pH decrease of more than 0.08 was only observed in three instances twice was 0.[illegible] once 0.13

(26) 103

	lactate (venous blood) mEq/Kg H_2O mean	range
precompression	1.50	0.72 to 2.10
compression (10 to 20 min)	1.83	1.00 to 2.40
compression (20 to 40 min)	1.50	1.05 to 2.62
postcompression	1.33	0.87 to 2.18

(27) 110

precompression value minus postcompression value

protein g/Kg H_2O	pH	Na	K	cations	Cl HCO_3	lactate	(cations minus Cl HCO_3+lactat)
			m E q / K g H $_2$ O				
	0.1	0.0	-0.40	-0.40	-0.06	0.33	0.23

22 78

mpl		N	Cl			
		flame	potent tit		V lhard	
containe	hours aft start dialy i	mEq/L	mEq/L	N minus Cl	mEq/L	N minus Cl
I	0	153.2	153.2	0.0	153.3	-0.1
	16	153.7	153.8	-0.1	155.4	1.7
	24	153.7	153.6	0.1	152.5	1.2
	40	153.6	154.4	0.8	153.3	+0.3
II	0	151.2	153.2	0.0	153.7	+0.5
	16	151.2	153.2	0.0	153.5	-0.3
	24	152.9	153.2	-0.3	152.1	+0.8
	40	153.1	153.4	0.3	152.7	+0.4
III	0	154.7	154.8	0.1	154.2	+0.5
	16	154.7	154.4	+0.3	154.8	0.1
	24	155.1	155.2	-0.1	153.8	1.3
	40	154.6	155.0	0.4	153.2	1.4
IV	0	152.6	152.4	+0.2	150.5	2.1
	16	152.5	152.6	-0.1	150.9	1.6
diff rence between N and Cl conc			mean	0.13		+0.57
			range	+0.3 to 0.8		2.1 to 1.7

23 98

subject and sampl	protein g/Kg H_2O	alb	α_1 gl	α_2 gl	β gl	fib γ-gl
		percentage of total prot i				
De 1	72.7	58.7	2.7	8.1	7.2	21.3
2	78.2	58.2	3.1	8.5	6.8	21.4
3	83.1	58.3	3.5	8.4	6.9	21.8
4	87.7	58.8	3.3	8.6	7.2	21.2
5	112.2	58.0	3.5	10.0	7.0	21.6
6	112.2	58.0	4.3	10.0	6.7	20.1
7	88.1	58.6	3.4	8.9	7.2	21.7
8	77.1	58.1	3.9	8.2	6.6	21.1
9	73.7	59.2	4.0	8.9	6.8	21.1
Be 1	67.8	62.2	3.4	7.1	7.2	20.1
2	67.8	60.4	3.9	7.1	7.8	21.0
3	80.1	60.4	3.7	7.0	7.6	21.3
4	92.7	60.7	3.5	7.6	7.3	20.9
5	106.2	59.8	3.7	7.9	8.4	20.1

(30) 127

SD of single determination of Δcation, Δanion and Δ(cat min $Cl+HCO_3$)

(valid for comparison of results obtained from different containers)

the values are calculated for dialysis units containing 63 g/Kg H_2O protein

SD in mEq/100 g protein

ΔNa	$\frac{100}{63}\sqrt{(0.03 \quad 0.11)^2 \quad 0.28^2}$	0.04
ΔK		0.06
ΔCa+Mg		0.10
ΔCl		0.03
ΔHCO_3		0.33
Δ(cat min $Cl+HCO_3$)		0.9[illegible]

(31) 128

difference between values obtained for

'distilled water' and for dialysis solution (first sample taken)

	pairs of samples	mean difference	SD of the difference for single pair	range of difference
pH	73	0.03	03	-0.10 to 0.13
			mEq/Kg H_2O	
Na	73	-0.02	0.1	-0. to 0.
K	73	-0.001	0.02	-0.06 to 0.07
Ca Mg	73	-0.001	0.02	-0.14 to 0.04
Cl	73	-0.04	0.16	1.7 to 0.4
HCO_3	7	-0.01	0.27	-0.8 to 0.7
(cat min $Cl+HCO_3$)	71	03	0.42	-0.7 to 1.7

(28) 125

difference between value obtained from
two samples f the sam dialysi solution
(sampl taken first in sequenc minu sampl taken second)

	pairs of sampl	mean difference	SD of the difference for ingle pair	range f differenc
pH	22	0.003	0.01	-0.02 to 0.04
			m E q / K g H $_2$ O	
Na	21	0.08	0.28	0.6 to 0.8
K	22	0.01	0.030	-0.04 to 0.12
Ca Mg	22	-0.01	0.025	0.08 to 0.03
Cl	23	0.01	0.20	-0.4 (0.5
HCO_3	22	0.03	0.22	0.6 t 0.5
(cat mi Cl HCO_3)	22	0.03	0.40	0.7 to 0.6

(29) 126

diff renc between value obtained from
tw serum containing dialysis unit (same containe)
(sampl taken first in sequenc minu sampl taken second)

	pairs f samples	mean difference	SD f the diff rence for singl pair	ange of differenc
pH	48	-0.003	0.01	-0.03 0.03
		m E q / 1 0 0 g	p	t l
ΔN	34	0.03	0.81	1.7 to 2.2
ΔK	34	0.008	0.08	-0.05 to 0.11
ΔCa Mg	34	0.010	0.18	-0.32 to 0.24
ΔCl	34	-0.18	0.77	1.8 to 1.9
ΔHCO_3	33	-0.03	0.44	-0.8 to 1.0
Δ(cat min Cl HCO_3)	33	0.21	0.80	1.7 to 2.0

Not the actual protein concentrations of the serum samples ranged from 52 to 75 g/Kg H_2O the m an value being 63 g/Kg H_2O

dialysis solution		pH	Na	K	Ca+ Mg	Cl	HCO_3	inorg phos	osm cal	osm [illegible]	[illegible]
			m E q / K g H_2O								
before dialysis		[illegible]	147,7	[illegible]	4,3	[illegible]	[illegible]	1,78	[illegible]	[illegible]	-0,3
after dialysis											
container I	sample 1	7,91	147,	[illegible]	[illegible]	[illegible]	[illegible]	1,74	[illegible]	[illegible]	[illegible]
	sample 2	7,91	147,5	[illegible]	[illegible]	[illegible]	[illegible]	1,74	[illegible]	[illegible]	-0,1
container II	sample 1	[illegible]	147,1	[illegible]	[illegible]	[illegible]	[illegible]	1,73	[illegible]	[illegible]	[illegible]
	sample 2	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

samples	pH	protein g/Kg H_2O	inorg. phos. mEq/Kg H_2O
dialysis sol.	7,73	—	[illegible]
serum	7, 4	[illegible]	[illegible]
dialysis sol.	7, 2	—	[illegible]
serum	7,12	[illegible]	[illegible]
dialysis sol.	[illegible]	—	1,30
serum	[illegible]	[illegible]	[illegible]
dialysis sol.	7,04	—	[illegible]
serum	7,02	[illegible]	[illegible]
serum	[illegible]	[illegible]	[illegible]
dialysis sol.	7,15	—	[illegible]
serum	[illegible]	[illegible]	[illegible]
serum	[illegible]	[illegible]	[illegible]
dialysis sol.	7,71	—	[illegible]
serum	7,73	[illegible]	[illegible]
serum	7,73	[illegible]	[illegible]

(32) 128

samples in thei sequence	E p I pH	CO_2 mEq/L	P_{CO_2} mm Hg	Exp II pH	Exp III pH
1 dialysis solution	7.72	33.8	27	7.09	7.81
2 'distilled wat	7.72	33.8	27	7.10	7.51
3 distilled wat	7.72	33.6	27	7.05	7.51
4 'distilled wate	7.72	33.8	27	7.09	7.58
5 dialy is solution	7.73	33.7	27	7.08	7.51
6 'distilled water	7.74	33.9	25	7.10	7.52
7 dialysi solution	7.72	34.2	27	7.08	7.81
8 'distilled wat	7.72	33.8	27	7.08	
9 dialysis solution	7.73	33.7	26	7.08	

(33) 133

	protein dialysi mts	ΔNa		ΔCa Mg		$\Delta^{(\ \ t\ \ ml}_{Cl\ HCO_3)}$	
		pl	___	pla	se	pl	___
	g/Kg H_2O	m E q / 1 0 0 g p t i					
dialysis xp I	85 to 89	9.1	9.2	2.74	2.71	17.1	13.4
		8.1	8.1	2.63	2.68	16.5	16.3
dialysis xp II	83 to 87	8.8	8.4	2.22	2.08	13.3	13.9
		8.4	8.0	2.25	2.04	15.1	14.2
	mean valu	8.8	8.9	2.47	2.36	16.0	15.5

(34) 135

d i l t d m m p l			also obtained with u d i l t d serum mieu valu obtained with d i l d serum			
protein oncentr tion			ΔCl		$\Delta^{(\ \ \ \ min}_{Cl\ HCO_3)}$	
range	mean	numbe	mean difference	2 SE	mean diff rence	2 SE
g/Kg H_2O			m E q / 1 0 0 g p i			
40 to 50	43.8	9	-0.98	0.74	0.44	0.77
30 t 40	35.7	18	0.86	0.68	0.78	0.71
20 to 30	26.5	5	0.28	1.71	0.22	1.80
10 to 20	18.8	6	2.18	2.40		
	18.3	5			3.40	2.80

Not for one of th mples in the protein range (10 20) HCO_3 was no det rmined

dialysis solution		pH	Na	K	Ca Mg	Cl	HCO_3	inorg phos	sum cat	sum an	cat minus an
			m Eq / K g H$_2$O								
before dialysis		[illegible]	147,7	[illegible]	4,37	[illegible]	[illegible]	1,76	[illegible]	[illegible]	[illegible]
after dialysis											
container I	sample 1	7,91	147,	[illegible]	[illegible]	[illegible]	[illegible]	1,74	[illegible]	[illegible]	[illegible]
	sample 2	7,91	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	1,74	[illegible]	[illegible]	[illegible]
container II	sample 1	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	1,73	[illegible]	[illegible]	[illegible]
	sample 2	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

sample	pH	protein g/Kg H$_2$O	inorg phos mEq/Kg H$_2$O
dialysis sol.	[illegible]	—	1,80
serum	7,74	[illegible]	[illegible]
dialysis sol.	[illegible]	—	[illegible]
serum	[illegible]	[illegible]	[illegible]
dialysis sol.	[illegible]	—	[illegible]
serum	[illegible]	[illegible]	[illegible]
dialysis sol.	[illegible]	—	[illegible]
serum	[illegible]	[illegible]	[illegible]
serum	[illegible]	[illegible]	[illegible]
dialysis sol.	[illegible]	—	[illegible]
serum	[illegible]	[illegible]	[illegible]
serum	[illegible]	[illegible]	[illegible]
dialysis sol.	7,71	—	[illegible]
serum	7,73	[illegible]	[illegible]
serum	7,73	[illegible]	[illegible]

(32) 128

samples in th l sequence	Exp I pH	Exp I CO_2 mEq/L	Exp I P_{CO_2} mm Hg	Exp. II pH	Exp III pH
1 di ly l solution	7,72	33,8	27	7,09	7,51
2 'distilled wat	7,72	33,8	27	7,10	7,51
3 'distilled w ter	7,72	33,8	27	7,09	7,51
4 'distilled wat	7,72	33,8	27	7,09	7,50
5 dialysi sol tion	7,72	33,7	27	7,08	7,51
6 distilled wat	7,74	33,8	25	7,10	7,52
7 dialy is solution	7,72	34,2	27	7,08	7,51
8 'distilled wat	7,72	33,8	27	7,08	
9 dialysis solution	7,73	33,7	28	7,08	

(33) 133

	prot in dialysis units g/Kg H_2O	ΔNa pl	ΔNa se	ΔCa Mg pla	ΔCa Mg se	Δ_{Cl} (min HCO_3) pl	Δ_{Cl} (min HCO_3) se
		m Eq / 100 g p				t	i
dialysi exp. I	65 to 69	9.1	9.2	2.74	2.71	17.1	15.4
		8.1	8.1	2.03	3.08	16.5	16.2
dialysi xp II	63 to 67	8.5	8.4	2.22	2.08	15.3	15.9
		8.4	8.6	2.25	2.04	15.1	14.2
mean value		8.8	8.9	2.47	2.36	16.0	15.8

(34) 135

dil t d m mpl protein concentration range g/Kg H_2O	mean	numbe	value obtained with u dil ed serum minus valu obtained with dil t d serum ΔCl mean differenc	2 SE	Δ_{Cl+HCO_3} (o min) mean difference	2 SE
			m Eq / 100 g p			
40 to 50	43.8	9	0.08	0.74	0.44	0.77
30 to 40	35.7	18	-0.86	0.68	0.78	0. 1
20 to 30	25.5	5	0.28	1.71	-0.22	1.80
10 to 20	16.8	6	3.18	2.40		
	16.3	5			3.40	2.80

Not fo one of the sampl in the prot in ange (10 to 20) HCO_3 no determined

PROTEIN COMPOSITION

Protein composition	SA (mEq/kg H_2O)	NCE (mEq/100 g prot)	Na 'free' (mEq/kg H_2O)	Na bound (mEq/100 g prot)	Ca 'free' (mEq/kg H_2O)	Ca bound (mEq/100 g prot)	Mg 'free' (mEq/kg H_2O)	Mg bound (mEq/100 g prot)	SA (mEq/kg H_2O)	NCE (mEq/100 g prot)	Ca 'free' (mEq/kg H_2O)	Ca bound (mEq/100 g prot)
	(not yet corrected for [illegible] errors)								(corrected for pH error)			
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

date	subject	age	number of samples	protein intake	pH plasma arterial	pH plasma calculated	water content plasma	equation	number of samples	PLASMA CONCENTRATION: prot g/L	Na	K	Ca	Mg	Cl	HCO_3	lactate
[illegible]																	
[illegible]																	
[illegible]																	
[illegible]																	
[illegible]																	
[illegible]																	
[illegible]																	
[illegible]																	
[illegible]																	
[illegible]																	
[illegible]																	
[illegible]																	
[illegible]																	
[illegible]																	
[illegible]																	
[illegible]																	
[illegible]																	
[illegible]																	
[illegible]																	
[illegible]																	
[illegible]																	
[illegible]																	
[illegible]																	
[illegible]																	
[illegible]																	
[illegible]																	
[illegible]																	
mean value					[illegible]		[illegible]			[illegible]	[illegible]	[illegible]			[illegible]	[illegible]	[illegible]
maximum value					[illegible]					[illegible]	[illegible]	[illegible]			[illegible]	[illegible]	[illegible]
minimum value					[illegible]						[illegible]					[illegible]	

after [illegible]

date	subject																
[illegible]																	
[illegible]																	
[illegible]																	
[illegible]																	
[illegible]																	
[illegible]																	

	[illegible]								
	protein g/L plasma	pH [illegible]	Na	K	Ca	Mg	Cl	HCO_3	inorg phos
			m Eq / L						
experimental result									
number observations	(28)	(28)	(28)	(28)	(27)	(27)	(28)	(28)	(30)
mean value	68	7.38	138	3.85	4.85	[illegible]	104.0	28.1	1.0
maximum value	76.8	7.40	[illegible]	4.32	[illegible]	1.86	109.6	30.5	2.1
minimum value	61.3	7.31	[illegible]	3.38	4.3	[illegible]	101.7	21.2	1
correction mean value for error a)			−0.4	0.1					
d)		0.03						0. 0.	
best estimate of mean value	68.2	7.39	[illegible]	[illegible]	4.85	[illegible]	104.0	28.1	1.0

protein composition (mean values in % of total protein)

[illegible] (n 27) alb 57 [illegible] glob 18 [illegible] glob 25.8

electrophoresis (n=28) alb [illegible] α_1 [illegible] α_2 [illegible] β [illegible] γ glob [illegible]

[illegible] error a) ... d) etc. refer to the section on systematic errors in chapter V

	[illegible]						
	pH endocellular	Na free mEq per Kg H_2O	Na bound mEq per 100 g prot	Ca free mEq per Kg H_2O	Ca bound mEq per 100 g prot	Mg free mEq per Kg H_2O	Mg bound mEq per 100 g prot
experimental result							
number observations	(28)	(28)	(28)	(27)	(27)	(27)	(27)
mean value		40.3		[illegible]	2	[illegible]	[illegible]
maximum value	[illegible]	[illegible]	[illegible]	[illegible]	3.1	[illegible]	
minimum value	[illegible]			[illegible]		[illegible]	[illegible]
correction mean value for error)		−0.2	−0.2				
d)	[illegible]	[illegible]*	[illegible]*	−0.09	[illegible]	−0.03*	[illegible]*
best estimate of mean value	34	130.0	[illegible]*	2.75 −0.13*	[illegible]*	[illegible]	[illegible]*

Note: the corrections marked with * are very much an estimate; for their [illegible] the reader is referred to chapter IV

(38) 149

valu obtained when the regression line i alculated from

subject	all samples RA	all samples NCE	compre ion sampl only RA	compre ion sampl only NCE
	mEq per Kg H_2O	mEq pe 100 g prot	mEq pe Kg H_2O	mEq pe 100 g prot
Gee	3,6	16,8	4,6	17,8
Ott	6,7	17,4	5,5	17,6
dGe (I)	11,5	13,6	13,0	12,0
Off	7,1	16,3	6,0	17,3
Ren	4,6	16,9	6,8	14,9
dV (I)	7,0	14,1	4,9	16,6
dGe (I)	5,3	18,7	4,6	18,4
dR	6,9	18,4	4,2	20,0
dLo	2,4	18,4	3,1	18,7
Vre	6,2	17,3	6,0	15,0
Le (I)	4,4	17,2	3,5	18,0
Bir	6,0	18,0	4,4	18,6
Poo	4,7	17,0	2,8	18,8
Lin	2,8	17,8	1,8	18,0
Hen	4,1	18,2	2,6	20,4
Dow	7,5	16,2	5,6	17,9
Bee	6,1	16,9	2,8	19,1
mean valu	5,6	17,0	5,0	17,7

(39) 152

rror caused by addition of heparin

heparin solution added t samples	N plasma conc.	free	RA	N bound	NCF
	mEq/Kg H_2O			mEq/100 g prot in	
0,01 ml	+0,2	+0,1	+0,1	+0,1	+0,
0,03 ml	+0,6	+0,3	+0,3	+0,3	+0,3

	protein g/L plasma	pH	Na	K	Ca	Mg	Cl	HCO_3	inorg phos
			m E q / L						
experimental result									
number observations	(28)	(28)	(30)	(30)	(27)	(27)	(28)	(28)	(30)
mean value	68.2	7.38	171.3	3.83	4. 5	[illegible]	104.8	26.1	1.0
maximum value	76.8	7.40	1 3	4.32	4.83	[illegible]	108.8	28.5	2.5
minimum value	8	7.3	38	3.3	4.3	48	1 L	23.8	1.4
correction mean value for error a)			−0						
b)			0	0					
)									
d)		0.03						0	
)								0	
f)									
best estimate of mean value	68.2	7.3	0	96	4. 5	1	104.8	26.1	1

protein composition (mean values in % of total prot in)

[illegible] (n 7) Alb [illegible] -glob 18.4 [illegible]-glob 28.8

electrophoresis (n 20) Alb 58.8 α_1 [illegible] α [illegible] 7 L [illegible] β-glob 18

Note errors a))) d) [illegible] refer to the section on systematic errors in chapter

	pH	Na free	Na bound	Ca free	Ca bound	Mg free	Mg bound
		mEq per Kg H_2O	mEq per 80 g prot	mEq per Kg H_2O	mEq per 80 g prot	mEq per Kg H_2O	mE per 100 g prot
experimental results							
number observations	(28)	(30)	(28)	(27)	(27)	(27)	(27)
mean value	1	68	0	2.94	2.78	78	0.83
maximum value		42	1	7	2.1	1.3	
minimum value	77	8		2.64	2	0. 3	0
correction mean value for error)		−0.2	−0				
)			0				
		−0.2*	0.7*	−0.09	0.07	−0.03	0.03*
)	03						
	—	3.3	3.3	−0.10*	13*	−0.03	3*
best estimate of mean value	34	38	13	2.78	2.85	L	0.90

Note the corrections marked with * are very much an estimate for their argumentation the reader is referred to chapter IV

(42) 153

m l al f RA d NCE

	pH endcompr	RA mEq pe Kg H_2O	NCE mEq per 100 g prot
experimental result			
numbe observations	(26)	(28)	(28)
mean value	7,31	8,2	18,4
maximum valu	7,35	11,5	20,2
minimum value	7,27	2,8	12,2
correction mean value for rror)		−0,2	−0,2
b)			0,3
)		0,8	0,7
d)	0,03	−0,1	
)		0,1	0,3
f)	—	—	—
best estimate of m an value	7,34	8,0	12,8

(43) 154

m p l f lt bt i d f C d M g
i 1961 d i th p t t dy

		numbe observ.	protein g/Kg H_2O	cation in plasma mEq pe Kg H_2O	ation free mEq per Kg H_2O	ation bound mEq per 100 g prot	free ation percentage plasma conc.
Ca	1961	18	71,0**	5,02	2,92	2,98	58
	present study	37	72,9	4,95	2,94	2,78	59
Mg	1961	18	71,0**	1,78	1,15	0,80	64
	present study	27	72,8	1,79	1,20	0,83	67

Not van LEEUWEN et al. (1961)

** protein concentration 3 % too low because f rror in macro-Kjeldahl determination (see ch pt III)

(44) 154

normal subjects	plasma Na (mEq/L)	plasma K (mEq/L)	plasma RA (mEq per Kg H_2O)	plasma ACE (mEq per 00 g prot)	[illegible] plasma Na (mEq/L)	[illegible] plasma K (mEq/L)	[illegible] plasma RA (mEq per Kg H_2O)	[illegible] plasma ACE (mEq per 00 g prot)
vLa (f)	77	4.09	7.7	1 4	72	19	8.8	12.4
Gee	37	3.8	8.8	6.0	179.8	83	8	3.9
Ott	179.8	70	7	4	142	60	8.3	8.6
dOe (f)	128.3	3.90	1.8	3.0	14	3.68	1	1
Off	1	3.90	1	10.3	42.8	3.90	7	18
dT (f)	38	3.37	4	10.2	1 1	3.83	8	28
Lie	136.7	3.76	6.7	1	728	3.67		8
vdW	137.8	90	3	3	367	2	3	3.1
La (f)	134.0	3.63	8	8.1	78	3.77	7	1
Jan	38.8	3.88	4.3	20.3	1	3.37	8	31
Ce	48	4.00	3.3	9 1	3.7	4.13	4	22
re (f)	138.0	3.70	8	16	1 12	3.09	6	16.7
vdP	140.0	3.78	6	14.3	1 78	02	16	1 0
vdB	38	3.73		8.0	1 6	87	8	17
mean value	1 88	3.78	8	8.3	1.1	3.00	7 8	8.3

(46) 155

patients	plasma Na (mEq/L)	plasma K (mEq/L)	plasma RA (mEq per Kg H_2O)	plasma NCE (mEq per 100 g prot)	[illegible] plasma Na (mEq/L)	[illegible] plasma K (mEq/L)	[illegible] plasma RA (mEq per Kg H_2O)	[illegible] plasma NCE (mEq per 00 g prot)
	38.8	4.3	8.1	18.8	38.3	4.16	6.3	8.3
Kr	136.0	6.60	1		37	3.63	2	10.0
Or	33.0	63	28.3	21	138.3	90	2	27.8
H	138.6	3.00	11.8	11.3	42.8	3.93	14.1	8.7
vM	13 3	93	9 3	10.1	13 8	3.07	28	12.0
vdW	28.8	3.33	3	8.3	137.6	59	21	8
mean value	13	3	8.1	18	137.6	4.54	8.3	10.1

for table 45 p t.o

date and subject	sex	age	diagnosis	number of samples	protein increase (%)	haemoglobin (g%)	pH plasma	PLASMA CONCENTRATIONS
[illegible]								
[illegible]	[illegible]	[illegible]	myeloma (γ-type)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	myeloma (γ type)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	myeloma (γ-type)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	myeloma (β type)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	myeloma (γ type)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	myeloma (β type)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]								
[illegible]	[illegible]	[illegible]	liver cirrhosis	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	chronic infection	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	chronic infection	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	liver cirrhosis	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	liver cirrhosis	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	Whipple disease	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]								
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]								
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

[illegible]

PROTEIN COMPOSITION

(not yet corrected for systematic errors)

corrected for pH error

[illegible]

date	subject	pH	mM CH₃HCO₃ (mM/kg per g/l)	pH	mM HCO₃ (m)	Na	K	Ca/Mg	Cl	HCO₃	Number (daily)	pH	prot (g/kg)	HCl	ΔNa	Δ	Δ^{C}_{Mg}
Normal																	
[illegible]	Le (I)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	J	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	ra (I)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	sd	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	vdB	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	vLe (I)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	pool I	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	Oma	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	Le (II)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	Dem	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	vLe (II)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	vLe-Le	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	dV	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	vdW	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	dOe	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	dBe	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	dLa	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	rs	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	br	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	ra (II)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	pool II	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	pool III	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	pool IV	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Patients																	
[illegible]	Fm — male, myeloma (γ-type)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	vO — male, 73 yr, macroglobulinemia	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	Fo (I) — male, 67 yr, myeloma (γ-type)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	Fo (II)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	La — female, 60 yr, myeloma γ	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

date	subject	pH	ext mEq Cl Na	pH	ext mEq Cl HCO_3	Na	K	Mg	Cl	HCO_3			pH	prot		Δ Na	Δ	$Δ^C_{Mg}$
[illegible]	Le II	[illegible]																
[illegible]	[illegible]	[illegible]																
[illegible]	[illegible]	[illegible]																
[illegible]	[illegible]	[illegible]																
[illegible]	[illegible]	[illegible]																
[illegible]	vLe II	[illegible]																
[illegible]	pool I	[illegible]																
[illegible]	Ons	[illegible]																
[illegible]	Le II	[illegible]																
[illegible]	Dus	[illegible]																
[illegible]	vLe II	[illegible]																
[illegible]	vLe-Le	[illegible]																
[illegible]	dV	[illegible]																
[illegible]	vdV	[illegible]																
[illegible]	dOv	[illegible]																
[illegible]	dRe	[illegible]																
[illegible]	Le	[illegible]																
[illegible]	re	[illegible]																
[illegible]	Ri	[illegible]																
[illegible]	re II	[illegible]																
[illegible]	pool II	[illegible]																
[illegible]	pool III	[illegible]																
[illegible]	pool IV	[illegible]																

Patients

[illegible]	[illegible]	male	[illegible] yr	myeloma	γ type [illegible]
[illegible]	vO	male	[illegible] yr	macroglobulinaemia	
[illegible]	Po II	male	[illegible] yr	myeloma	γ type [illegible]
[illegible]	Po II				
[illegible]	Le	female	[illegible] yr	myeloma	γ type

WATER

date	subject	pH	ml m CH_3COOH (ml/kg, per γ)	pH	ml max HCO_3 (m)	K	Ca / Mg	Cl	HCO_3			pH		HCl	Δ	Δ	
[illegible]	vLa III	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	J	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	vLa II	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	pool I	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	Om	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	vLa III	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	Dm	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	La III	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	vLa-La	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	dV	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	dLa	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	pool II	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	pool III	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	pool IV	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

P a t i e n t s

date	subject	sex / age	diagnosis	data
[illegible]	[illegible]	male	myeloma (γ type)	[illegible]
[illegible]	[illegible]	male, [illegible] yr	macroglobulinaemia	[illegible]
[illegible]	[illegible]	male, [illegible] yr	myeloma (γ type)	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	La	female, [illegible] yr	myeloma (γ type)	[illegible]

b [illegible] tl [illegible] l pH [illegible] g 7.10 7.50
(m [illegible] al [illegible])

		pH	Na dial. sol. mEq per Kg H_2O	Na Δ mEq per 100 g prot	K dial. sol. mEq per Kg H_2O	K Δ mEq per 100 g prot	Ca Mg dial. sol. mEq per Kg H_2O	Ca Mg Δ mEq per 100 g prot
serum	14	7.37	1 2.0	8.8	4.34	0.71	4.06	2.44
Na and K corrected for flame photometer error:			142.9	8.2	4.34	0.50		

pH range	I: Ca Mg dialysis solution, mean 4.0[illegible] mEq/Kg H_2O, range 4.00 to 4.25	pH mean	Δ Ca Mg mean mEq per [illegible] g prot	II: Ca+Mg dialysis solution, mean 4.00 mEq/Kg H_2O, range 3.47 to 4.32	pH mean	Δ Ca+Mg mean mEq per 100 g prot
6.50 to 6.75	4	6.60	2.06	8	6.56	2.03
[illegible] to [illegible]00	6	6.83	2.33	9	6.85	2.30
7.00 to 7.25	8	7. 1	2.01	8	7.11	2.01
[illegible]25 to [illegible]50	8	7.36	2. 1	18	7.36	2.35
7.50 to 8.00	8	7.64	[illegible]	9	7.60	4.50

(48) 166

b tl l pH g 7 10 – 7 50

	numbe observ	pH mean value	NCP mean valu mEq pe 100 g prot
plasma	3	7.39	17.9
serum	14	7.33	16.4
correction flame photomet rre			0.3
correction for arbamino-protein			0.3
erum corrected	14	7.33	17.0

mean protein concentration 62.3 g/Kg H_2O

(49) 166

dat	serum	pH range		regression line NCP on pH
3- 4-61	pool I	6.35 to 7.60	3	8.36 (pH 5.56)
18-12-61	pool II	6.78 to 7.37	3	11.06 (pH 5.59)
21-12-61	pool III	6.89 to 7.39	3	8.88 (pH 5.77)
2- 1-62	pool IV	6.99 t 7.98	3	5.39 (pH 4.41)
19- 4-61	Le (R)	6.68 t 7.39	3	7.27 (pH 5.20)
3- 5-61	Don	6.65 to 7.42	2	11.95 (pH 6.91)
8- 5-61	Le (M)	6.78 to 7.28	2	12.40 (pH 6.28)
14- 5-61	Le Le	6.66 to 7.36	3	10.08 (pH 5.88)
18- 5-61	dV	6.96 t 7.70	3	11.55 (pH 5.96)
24- 5-61	vdW	7.08 t 7.74	3	7.25 (pH 4.97)
29- 5-61	dOe	7.14 to 7.65	3	17.36 (pH 6.42)
1- 6-61	dR	6.82 to 7.46	3	8.76 (pH 4.72)
8- 6-61	dLa	6.33 t 7.15	3	8.22 (pH 6.02)
13- 6-61	Vre	6.82 to 7.37	3	8.39 (pH 5.23)
19- 6-61	Bir	6.76 to 7.28	3	11.16 (pH 5.74)
	g g	1 11		10.01 (pH 6.70)

Note- indicat the number of point from which the regression line was calculated.

1 1 pH [illegible] 7.10 7.80
(m[illegible] al[illegible])

		pH	Na dial. sol.	Na Δ	K dial. sol.	K Δ	Ca Mg dial. sol.	Ca Mg Δ
			mEq per Kg H_2O	mEq per 100 g prot	mEq per Kg H_2O	mEq per 100 g prot	mEq per Kg H_2O	mEq per 100 g prot
serum	14	7.33	142.9	8	4.34	0.71	4.06	2.44
Na and K corrected for flame photometer error:			142.9	8.2	4.34	0.76		

pH range	I: Ca+Mg dialysis solution, mean 4.03 mEq/Kg H_2O, range 4.00 to 4.20			II: Ca+Mg dialysis solution, mean [illegible] mEq/Kg H_2O, range [illegible] to [illegible]		
		pH mean	Δ Ca Mg mean mEq per 100 g prot		pH mean	Δ Ca+Mg mean mEq per 100 g prot
6.50 to 6.75	4	6.64	2.00		6.64	2.03
6.75 to 7.00	6	6.93	2.32	9	6.83	2.23
7.00 to 7.25		7. 1	2.9	8	7.11	2.8
.25 to .50	10	.30	2.41	10	7.33	3.06
7.50 to 8.00	6	7.64	4.03	9	7.00	4.58

numbe observ.	pH (inside) range	pH (inside) mean	ion	concentration dialyed sol. [C] [An] mEq/Kg H_2O	t ti 'distilled wat mean	2 SE	ti (R_{cat}, R) serum mean	2 SE
			Na	142.2	1.0001	0.0006	0.9363	0.0017
			K	4.21	0.9989	0.0021	0.9610	0.0048
21	7.20 to 7.75	<u>7.46</u>	C Mg	4.01	0.9980	0.0018	0.6010	0.0096
			Cl	120.6	0.9984	0.0014	0.9782	0.0030
			HCO_3	28.9	1.0021	0.0068	0.9813	0.0088
			N	142.6	1.0001	0.0003	0.9640	0.0022
			K	4.48	0.9992	0.0019	0.9708	0.0044
21	6.80 to 7.20	<u>6.91</u>	C Mg	4.11	1.0014	0.0011	0.7027	0.0077
			Cl	128.4	1.0002	0.0005	0.9920	0.0032
			HCO_3	23.7	0.9980	0.0041	0.9811	0.0058

<u>Not</u> all serum dat from table 47 re included except those where pH inside was not me sured (3) and those where the pH wa ou ide the ge indic ted above (2) The concentration ratio refe to protein concentration of 70 g/Kg H_2O the actu l prot in concent ation wa on the ave ge 82.8 g/Kg H_2O f the observation in th high pH g and 80.4 g/Kg H_2O for hose in the low pH range No correction has been mad for systematic rro

pH range	ion	R_{ext} uncorrected	R_{ext} corrected for [illegible] photometer error
7.30 to 7.75	Na	0.9543	0.9337
	K	0.961	0.94
6.50 to 7.30	Na	0.9644	0.9641
	K	0.971	0.95

plasma	pH inside	dialysis solution Ca (mEq/Kg H_2O)	dialysis solution Mg (mEq/Kg H_2O)	plasma ΔCa (mEq/100 g prot)	plasma ΔMg (mEq/100 g prot)	R_{Ca} (prot 70 g / Kg H_2O)	R_{Mg} (prot 70 g / Kg H_2O)
[illegible]	7.48	2.63	1.88	2.01	0.87	[illegible]	[illegible]
[illegible]	[illegible]	2.76	[illegible]	2.14	0.98	[illegible]	[illegible]
[illegible]	[illegible]	2.6	1.3	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	2.68	1.78	2.04	1.07	[illegible]	[illegible]
mean values	[illegible]	2.69	1.73	2.30	1.00	[illegible]	[illegible]

Note: pH dialysis solution

[illegible]

Note: [illegible]

date and protein preparations	pH	[illegible]	pH	[illegible]	Na	[illegible]	Mg	Cl	[illegible]	[illegible]	pH	[illegible]	[illegible]	Δ [illegible]	Δ	Δ
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

[illegible]

[illegible]	pH	[illegible]	pH	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	pH	[illegible]	[illegible]	Δ	Δ	Δ
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

[illegible]

[illegible]

[illegible]

CONCENTRATION RATIOS PROTEIN CONCENTRATION [illegible]

[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	Na	K	Ca+Mg	Cl	HCO_3	Na	K	Ca+Mg	Cl	HCO_3
[illegible]	[illegible]	[illegible]													
											[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
											[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]						[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
						[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
						[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		[illegible]	[illegible]
						[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		[illegible]	[illegible]
						[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		[illegible]	[illegible]
						[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		[illegible]	[illegible]
			[illegible]								[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
			[illegible]	[illegible]	[illegible]						[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
			[illegible]		[illegible]						[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
											[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

(56) 175

m l t f l b m

dat experiment	pH inside	alb/(alb glob) 100 M joer	lectr
16- 3-60	7.27	41.7	
21 9-60	7.39	44.6	
23- 3-61	7.32	46.6	48.5
26- 3-62	6.83	49.2	48.6
25-10-62	7.70		48.5
	7.00		47.4
	6.67		48.2

(57) 178

m l f pH

	pH inside	NCE m E
albumin	7.44 (8)	18.7 (8)
γ-globulin	7.47 (4)	2.2 (4)

(normal serum t pH 7.48 17.8

Not th numbe of xperiments from indicated betwee brackets these re discussed in the tex

(58) 179

l b

pH range	ion		pH inside
7.20 7.70	N Cl	8	7.44
6.60 to 7.20	Na Cl	7	6.80

Not th values are mean values and have Correction f K_M for the (approxima the values in the ase of γ globulin, but
at pH 7.44 0.942 0.944
at pH 6.80 0.953 0.937
The ion concentrations i the dialysi soluti with albumin ince these will be needed in ct

(59) 180

	in vivo ultrafiltration (plasma)	equilibrium dialysis (serum)
number of experiments	28	4
number of subjects	28	13
pH range	7.20 to 7.30	7.14 to 7.48
pH mean value corrected for systematic error	7.24	7.33
NCE mean value (mEq/100 g prot) corrected for systematic errors	17.8	17.0

(60) 180

	A in vivo ultrafiltration (plasma)		B equilibrium dialysis (serum)		A minus B
	pH	NCE mEq per 100 g prot	pH	NCE mEq per 100 g prot	difference in NCE after adjusting NCE of B to pH of A
Oes	7.34	[illegible]	7.44	18.1	3.7
Dam	[illegible]	[illegible]	7.43	18.1	-2.4
Le	7.32	[illegible]	[illegible]	17.4	1.5
dV	[illegible]	4.7	7.15	[illegible]	[illegible]
dGe	7.3	[illegible]	[illegible]	12.3	4.8
Re	[illegible]	[illegible]	[illegible]	10.9	1.5
Le	[illegible]	[illegible]	[illegible]	[illegible]	-0.8
Fe	[illegible]	7.4	[illegible]	[illegible]	2.9
B	[illegible]	[illegible]	[illegible]	[illegible]	-0.4
mean value	[illegible]		[illegible]		
correction for					
flame photometer error				[illegible]	
carbamino groups		[illegible]		[illegible]	
temperature blood	[illegible]				
[illegible]		[illegible]	7.[illegible]	[illegible]	

Note: the values listed for NCE under in vivo ultrafiltration are those obtained after correction for error due to pH change during [illegible] and for error caused by the addition of heparin.

(61) 182

		pH mean	Na free mEq per Kg H_2O	Na bound mEq per 100 g prot	Ca Mg free mEq per Kg H_2O	Ca Mg bound mEq per 100 g prot
in vivo ultrafiltration not corrected for Donnan equilibrium	[illegible]	[illegible]	[illegible]	[illegible]	4.02	2.76
equilibrium dialysis not corrected for Donnan equilibrium		7.33	[illegible]	[illegible]	4.0[illegible]	2.44
corrected for Donnan equilibrium	[illegible]	7.[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

56) 175

m l t r   o f   l b m l   d γ g l b l l

dat experiment	pH inside	$\frac{alb}{alb\ \ glob} \times 100$ M joor	lectr	NCE m E q / 1 0 0 g p t l calc	found	ΔN calc	found	ΔCa Mg calc	found
10-3-60	7.27	43.7		7.3	7.7	4.9	5.1	2.67	2.22
21-3-60	7.39	38.6		16.1	15.5	10.6	11.0	2.96	3.22
22-3-62	7.32	46.6	42.5	7.2	6.9	5.5	4.9	2.01	1.96
26-3-62	6.83	49.2	42.6	3.4	3.8	4.4	4.3	0.94	1.09
23-10-62	7.70		42.5	7.3	6.3	6.6	6.0	3.22	3.51
	7.00		47.4	3.7	4.2	3.5	3.6	1.76	1.92
	6.67		42.2	2.5	2.4	2.3	2.6	1.29	1.22

57) 178

m l f pH g 7.30 7.70

	pH inside	NCE m E q p 1 0 0 g p t l	ΔNa	ΔK	ΔCa Mg
Ibumi	7.44 (8)	19.7 (8)	11.8 (8)	0.25 (8)	4.80 (7)
γ globulin	7.47 (4)	2.2 (4)	0.5 (4)	0.05 (4)	1.05 (3)
(normal serum t pH 7.45		17.8	8.5	0.30	2.75)

Not the numbe f experiment from which the mean lue was calculated i indi ted between bra kets No correction was mad for systematic rors these re discussed in the text

58) 179

l b m l γ g l b l l

pH range	ion		pH inside	concentr dial sol mEq pe Kg H_2O	R_{cat} R_{an}		pH inside	R_{cat} R_{an}
7.20 7.70	Na	9	7.44	138.5	0.945	4	7.47	1.002
	Cl			118.2	0.981			1.010
6.60 to 7.20	N	7	6.90	142.7	0.928	4	6.94	1.005
	Cl			130.3	0.989			1.011

Not th value re mean values and have not been corrected fo syst m ti rer Correction of R_N for the (approximate) flam photomet error would not al th value in th ase f γ globulin, but for lbumin R_{Na} would become
at pH 7.44   0.942   0.944   (see discu sion
at pH 6.90   0.935   0.957   in the text)
The ion concentrations in the dialyzd solution are only given fo the xpe iment with albumin since these will be needed in chapt VIII

ACTA MEDICA SCANDINAVICA

SUPPLEMENTUM 421

SPECIFIC AND NONSPECIFIC IMMUNITY IN *CANDIDA* INFECTIONS

Experimental Studies of the Role of *Candida* Cell Constituents and Review of Literature

By

BOHDAN DOMAŃ

ACCOMPANIES VOL. 176

STOCKHOLM 1964

AMSTERDAM 1964
SCHELTEMA & HOLKEMA N.V

Department of Dermatology College of Physicians and Surgeons, Columbia University,
New York, New York

SPECIFIC AND NONSPECIFIC IMMUNITY IN *CANDIDA* INFECTIONS

Experimental Studies of the Role of *Candida* Cell Constituents and Review of Literature

By

Bohdan Dobias, M. D.

STOCKHOLM 1964

ACTA MEDICA SCANDINAVICA

has been published since 1919 as a continuation of Nordiskt Medicinskt Arkiv founded in 1869 by Axel Key The first volume of Acta Medica Scandinavica is therefore numbered LII (52)

The chief editors have been. Axel Key 1869 — 1900 C. G Santesson 1901 — 1915 I Holmgren 1916 — 1957 and Birger Strandell 1958 to date.

Acta Medica Scandinavica publishes original research papers in the field of internal medicine. Priority will be given to authors from countries which are represented on the editorial board Contributors from those countries should submit their papers to a representative of the country in qustion. Contributors from other countries should send their papers to the Editor at the address below

Submission of a manuscript for publication will be held to imply that the paper has not been published elsewhere and that, if accepted it will not be republished in any other journal in the same or similar form, without the Editor s written consent.

Papers will be published in English, French or German at the authors choice, followed by a summary in English.

The manuscripts shall be in final form as submitted. They shall be typewritten with double spacing and broad left-hand margin.

If a paper exceeds 16 printed pages, the cost for the excess pages shall be borne by the author No paper shall exceed 24 printed pages.

Subscription

The annual subscription to the journal, covering two volumes, each of 6 numbers is 140 Sw crowns or U S $ 27 25 *including postage* in the Scandinavian countries and in Holland 120 Sw crowns.

Address for subscriptions and all communications

ACTA MEDICA SCANDINAVICA

P O. Box 2052 Stockholm 2

Requests for duplicate copies of numbers that have gone *astray can only be considered* if made within a fortnight of the receipt of the *following* number

Department of Dermatology College of Physicians and Surgeons, Columbia University
New York, New York

SPECIFIC AND NONSPECIFIC IMMUNITY IN *CANDIDA* INFECTIONS

Experimental Studies of the Role of *Candida* Cell Constituents and Review of Literature

By

Bohdan Dobias, M. D

STOCKHOLM 1964

ACTA MEDICA SCANDINAVICA

has been published since 1919 as a continuation of *Nordiskt Medicinskt Arkiv* founded in 1869 by Axel Key The first volume of Acta Medica Scandinavica is therefore numbered LII (52)

The chief editors have been. Axel Key 1869 — 1900, C. G Santesson 1901 — 1915, I Holmgren 1916 — 1957 and Birger Strandell 1958 to date

Acta Medica Scandinavica publishes original research papers in the field of internal medicine. Priority will be given to authors from countries which are represented on the editorial board Contributors from those countries should submit their papers to a representative of the country in question. Contributors from other countries should send their papers to the Editor at the address below

Submission of a manuscript for publication will be held to imply that the paper has not been published elsewhere and that, if accepted it will not be republished in any other journal in the same or similar form without the Editor s written consent.

Papers will be published in English, French or German at the authors choice, followed by a summary in English.

The manuscripts shall be *in final form as submitted. They shall be typewritten with double spacing and broad left-hand margin.*

If a paper exceeds *16* printed pages, the cost for the excess pages shall be borne by the author No paper shall exceed 24 printed pages.

Subscription

The annual subscription *to the journal covering two volumes*, each of 6 numbers is 140 Sw crowns or U S $ 27 25 *including postage* in the Scandinavian countries and in Holland 120 Sw crowns.

Address for subscriptions and all communications
ACTA MEDICA SCANDINAVICA
P O Box 2052 Stockholm 2

Requests for duplicate copies of numbers that have gone astray can only be considered if made within a fortnight of the receipt of the following number

TABLE OF CONTENTS

Printed in Sweden
KUNGL. BOKTRYCKERIET P. A. NORSTEDT & SÖNER
STOCKHOLM 1964

(I) INTRODUCTION

In spite of recent progress in the treatment of moniliasis it has become apparent that cases of the disseminated form of this disease are difficult to handle with the present therapeutic methods, including antifungal antibiotics (103) The incidence of generalized moniliasis may not be entirely negligible (17 72, 144) and epidemics of moniliasis may occasionally occur particularly among institutionalized young children (14 96, 103 142) The role of antibacterial antibiotics in the enhancement of the disease has often been stressed but never satisfactorily explained (30) The possible role of nonspecific resistance in this phenomenon has not been explored. For these reasons it appears worth while to examine the role of immunity in the pathogenicity of *Candida* in general and to investigate the applicability of immunologic methods for the protection against moniliasis and for the treatment of disseminated forms of the disease.

Although a voluminous serologic literature deals with taxonomic considerations and diagnostic tests (63 110, 125 131 132) comparatively little experimental work has been devoted to the study of immunization against moniliasis The reason may be that since natural immunity may not be clearly demonstrated following monilial infection a development of an anti-*Candida* vaccine may not have been considered promising. It has been known that high agglutinin and complement fixing titers often develop in the course of natural *Candida* infections (63, 104 125, 131 137) and following immunization (53 104 130, 137) but the reasons for the frequent lack of protection against challenge in such situations have remained obscure. The possible adverse effects of the endotoxin-like substance of *Candida* and of hypersensitivity on resistance to *Candida* infections has not been adequately studied. Elimination of the endotoxin-like substance and of sensitizing substances from experimental vaccines has not been tried.

A study of the relationship of the structure of *Candida* to its immunological properties may lead to a separation of an effective protective antigen from cell fractions with other functions. Although electron microscopic studies of *Candida* as well as some chemical studies of its cell constituents are available, only limited attempts at correlation of the many structural and chemical elements of the fungus have been made. Possible physiologic functions of the anatomical and chemical subcellular components have remained all but obscure

Data from such studies may not only be applicable to *Candida* but they may be useful for the understanding of similar problems in other fungal organisms with a similar anatomic structure in the expectation that similar principles may be used for immunization against these

(1) INTRODUCTION

In spite of recent progress in the treatment of moniliasis it has become apparent that cases of the disseminated form of this disease are difficult to handle with the present therapeutic methods, including antifungal antibiotics (105) The incidence of generalized moniliasis may not be entirely negligible (17 72, 144) and epidemics of moniliasis may occasionally occur particularly among institutionalized young children (14 26, 103, 142) The role of antibacterial antibiotics in the enhancement of the disease has often been stressed but never satisfactorily explained (30) The possible role of nonspecific resistance in this phenomenon has not been explored. For these reasons it appears worth while t examine the role of immunity in the pathogenicity of *Candida* in general and to investigate the applicability of immunologic methods for the protection against moniliasis and for the treatment of disseminated forms of the disease.

Although a voluminous serologic literature deals with taxonomic considerations and diagnostic tests (63 110, 125, 131 132) comparatively little experimental work has been devoted to the study of immunization against moniliasis. *The reason may be that since natural* immunity may not be clearly demonstrated following monilial infection a development of an anti-*Candida* vaccine may not have been considered promising. It has been known that high agglutinin and complement fixing titers often develop in the course of natural *Candida* infections (63, 104 125, 131 137) and following immunization (53, 104 130 137) but the reasons for the frequent lack of protection against challenge in such situations have remained obscure. The possible adverse effects of the endotoxin-like substance of *Candida* and of hypersensitivity on resistance to *Candida* infections have not been adequately studied. Elimination of the endotoxin-like substance and of sensitizing substances from experimental vaccines has not been tried.

A study of the relationship of the structure of *Candida* to its immunological properties may lead t a separation of an effective protective antigen from cell fractions with other functions. Although electron microscopic studies of *Candida* as well as some chemical studies of its cell constituents are available, only limited attempts at correlation of the many structural and chemical elements of the f ngus have been made. Possible physiologic functions of the anatomical and chemical subcellular components ha e remained all but obscure.

Data from such studies may not only be applicable to *Candid* but they may be useful for the understanding of similar problems in other fungal organisms with a simila anatomic structure in the expectation that similar principles may be used for immunization against these

(1) INTRODUCTION

In spite of recent progress in the treatment of moniliasis it has become apparent that cases of the disseminated form of this disease are difficult to handle with the present therapeutic methods, including antifungal antibiotics (105). The incidence of generalized moniliasis may not be entirely negligible (17, 72, 144) and epidemics of moniliasis may occasionally occur particularly among institutionalized young children (14, 26, 103, 142). The role of antibacterial antibiotics in the enhancement of the disease has often been stressed but never satisfactorily explained (30). The possible role of nonspecific resistance in this phenomenon has not been explored. For these reasons it appears worth while to examine the role of immunity in the pathogenicity of *Candida* in general and to investigate the applicability of immunologic methods for the protection against moniliasis and for the treatment of disseminated forms of the disease.

Although a voluminous serologic literature deals with taxonomic considerations and diagnostic tests (63, 110, 1 5, 131, 132), comparatively little experimental work has been devoted to the study of immunization against moniliasis. The reason may be that since natural immunity may not be clearly demonstrated following monilial infection, development of an anti-*Candida* vaccine may not have been considered promising. It has been known that high agglutinin and complement fixing titers often develop in the course of natural *Candida* infections (63, 104, 125, 131, 137) and following immunization (55, 104, 130, 137) but the reasons for the frequent lack of protection against challenge in such situations have remained obscure. The possible adverse effects of the endotoxin-like substance of *Candida* and of hypersensitivity on resistance to *Candida* infections have not been adequately studied. Elimination of the endotoxin-like substance and of sensitizing substances from experimental vaccines has not been tried.

A study of the relationship of the structure of *Candida* to its immunological properties may lead to a separation of an effective protective antigen from cell fractions with other functions. Although electron microscopic studies of *Candida* as well as some chemical studies of its cell constituents are available, only limited attempts at correlation of the many structural and chemical elements of the fungus have been made. Possible physiologic functions of the anatomical and chemical subcellular components have remained all but obscure.

Data from such studies may not only be applicable to *Candida* but they may be useful for the understanding of similar problems in other fungal organisms with a similar anatomic structure in the expectation that similar principles may be used for immunization against these

organisms and for the explanation of various aspects of their pathogenicity

The purpose of this paper is to report experimental results concerning the effects of cell constituents of *Candida albicans* prepared by mechanical disintegration of its yeast cells, as far as they have relation to immunization against and hypersensitivity to the fungus. Their effect on nonspecific resistance against other organisms will also be reported A review of literature was undertaken on specific and nonspecific immunity in experimental and human moniliasis, and on hypersensitivity in disseminated monilial infection another factor which appears to influence the resistance to the disease.

The applicability of principles of nonspecific resistance for the explanation of the apparent enhancement of moniliasis and of bacterial infections not susceptible to antibiotics during antibiotic treatment and after the use of various vaccines will be discussed in view of the finding that endotoxins of gram-negative organisms enhance under certain conditions the growth of *Candida* and of other organisms in vivo Methods directed towards neutralizing endotoxins will be mentioned as possible means of counteracting the enhancement of fungal and bacterial infections by antibiotics and the constant stimulation of growth of fungi by endotoxin like substances which may take place in systemic mycoses. The possible use of active and passive specific immunization for the protection against and treatment of moniliasis will be discussed. The applicability of the findings concerning the immunological properties of various cell constituents of *Candida* to other fungi will also be discussed.

The report on own experimental findings is followed rather than preceded by a critical review of experiments of other workers concerning the relation of allergy to immunity in moniliasis, and those dealing with immunizations, since it is believed that experimental results reported here particularly those concerning nonspecific resistance and hypersensitivity are helpful in evaluating the mechanism of action of past experimental findings in a new light

(2) EXPERIMENTAL

(a) Materials and Methods

Microorganisms. — Strain No. 1799.2h. of *Candida albicans* was used in all experiments. It as originally isolated in 1955 from the blood stream of patient with systemic moniliasis. For the preparation of intravenous inoculations the organism was grown on Sabouraud' glucose agar in Petri dishes for 48 hours at the temperature of 37 C Fresh cultures were prepared for each inoculation. The yeast cells were count ed in hemocytometer The same strain was grown for the production of ll frac tions in Roux bottles on Sabouraud' glucose agar at room temperature for 3 day

Salmonella enteritidis strain B 27 was kindly provided by Dr C. A. Slanetz from the Department of Animal Care, College of Physicians and Surgeons This strain was originally isolated from rabbit. A fresh cultur as grown for 24 hours at 37 C prior each challenge The organism was transferred each time by placing 0.2 c. of cultur material in 10 cc of nutrient broth.

Ext act from Microorgan ms — The endotoxin of *Escherichia oli* was a lipopol sac haride of *E. ol* 026 B6 obtained from h Dif Laboratories, Detroi It was prepared by the Boivin trichloracetic acid procedur as modified by Webster Sagin, Landy and Johnson (146)

Cell f actions of *Candida albicans* ere prepared from the fungus grown as de bed The grow h consisted almost ex lusiv l of east cells. The harvested cells er subsequently washed 5 times th ater They ere then mechanically disrupted in ll homogenizer described by Merkenschlager Schlossman and Kurz (86) and manufactured by B Braun, Melsungen, German T cuts ex of 50 % suspension of *Candida* cells in distilled water were added to 50 gm. of glass beads (Ballotini No. 31/8, diameter 0.5 mm., manufactured by Dragon-Werk Georg Wild, Bayreuth, Germany) in bottle with a capacity of 75 cc. The chamber in which this bottle was rotating eccentrically was cooled with liquid carbon dioxide. After a treatment lasting about 8—9 minutes an almost complete disintegration of the yeast cells was observed under the microscope where cell fragments of various sizes er seen. Counts on pour plates done on 2 batches of cell walls re ealed that one of about 100,000 cells was iable. The cell debris was then shaken for an hour and a half on a ibrator (Syntron Paper Jogger Type PJ 4) The suspension was kept in refrigerator overnight. The next day it was shaken again for 1 hour It was then centrifuged at 14,500 RPM (about 27,000×G) for 4 hours. The supernatant was filtered through an ultra-fine ground glass filter The filtrate which was clear straw-colored fluid was lyophilized, stored in efrigerator and econstituted with physiologic saline solution just before use.

The sediment as washed 5 times with ter lyophilized, stored for future use in refrigera or and resuspended at least 24 hours befor use in physiologic saline solution ontaining 0.01 % Merthiolate.

One mg. of cell walls was prepared f om approximately 250,000,000 cells, one mg. of the soluble extract from about 100,000,000 cells.

Animal — Male ht Swiss mic weighing 20—25 gm were housed 10 to metal cage fed commercial pellets (supplied by Hemlock Hollow Farm, Wayne, N J) and given ter *ad libitum*. Male guinea pigs eighing 180—220 gm. wer used in hypersensitivity experiments.

organisms and for the explanation of various aspects of their pathogenicity

The purpose of this paper is to report experimental results concerning the effects of cell constituents of *Candida albicans* prepared by mechanical disintegration of its yeast cells, as far as they have relation to immunization against and hypersensitivity to the fungus. Their effect on nonspecific resistance against other organisms will also be reported. A review of literature was undertaken on specific and nonspecific immunity in experimental and human moniliasis, and on hypersensitivity in disseminated monilial infection, another factor which appears to influence the resistance to the disease.

The applicability of principles of nonspecific resistance for the explanation of the apparent enhancement of moniliasis and of bacterial infections not susceptible to antibiotics during antibiotic treatment and after the use of various vaccines will be discussed in view of the finding that endotoxins of gram-negative organisms enhance under certain conditions the growth of *Candida* and of other organisms in vivo Methods directed towards neutralizing endotoxins will be mentioned as possible means of counteracting the enhancement of fungal and bacterial infections by antibiotics and the constant stimulation of growth of fungi by endotoxin like substances which may take place in systemic mycoses. The possible use of active and passive specific immunization for the protection against and treatment of moniliasis will be discussed. The applicability of the findings concerning the immunological properties of various cell constituents of *Candida* to other fungi will also be discussed.

The report on own experimental findings is followed rather than preceded by a critical review of experiments of other workers concerning the relation of allergy to immunity in moniliasis, and those dealing with immunizations, since it is believed that experimental results reported here particularly those concerning nonspecific resistance and hypersensitivity are helpful in evaluating the mechanism of action of past experimental findings in a new light.

(2) EXPERIMENTAL

( ) *Materials and Methods*

Microorganisms. — Strain No. 1799 2K of *Candida albicans* was used in all experiments. It was originally isolated in 1955 from the blood stream of patient with systemic moniliasis. For the preparation of intravenous inoculations the organism was grown on Sabouraud's glucose agar in Petri dishes for 48 hours at the temperature of 37 C. Fresh cultures ere prepared for each inoculation The yeast cells were counted in hemocytometer The same strain was grown for the production of ell fractions in Roux bottles on Sabouraud's glucose agar at room temperature for 3 days.

Salmonella enteritidis strain B 27n was kindly provided by Dr. C. A. Slanetz from the Department of Animal Care, College of Physicians and Surgeons. This strain was originally isolated from rabbit. A fresh culture as grown for 24 hours t 37 C prior to each challenge The organism as transferred each time by placing 0.2 cc. of culture material in 10 cc. of nutrient broth.

Extract from Microorganisms. — The endotoxin of *Escherichia coli* was lipopolysaccharide of *E. oli* 026 B6 obtained from the Difco Laboratories, Detroit. It was prepared by the Boivin trichloracetic acid procedure as modified by Webster Sagin, Landy and Johnson (146)

Cell fractions of *Candida albicans* were prepared from the fungus grown as described The growth consisted almost exclusively of yeast cells. The harvested cells ere subsequently washed 3 times with ater They were then mechanically disrupted in cell homogenizer described by Merkenschlager Schlossman and Kurz (86) and manufactured by B. Braun, Melsungen, Germany Twenty cc of 30 % suspension of *Candida* ells in distilled water were added to 50 gm. of glass beads (Ballotini No. 31/B, diameter 0.5 mm., manufactured by Dragon-Werk Georg Wild, Bayreuth, Germany) in bottle with capacity of 75 cc. The chamber in which this bottle was rotating eccentrically was cooled with liquid carbon dioxide. After a treatment lasting about 8—9 minutes an almost complete disintegration of the yeast cells was observed under the microscope where cell fragments of various sizes were seen. Counts on pour plates done on 2 batches of cell walls revealed that one of bout 100,000 cells was viable. The cell debris was then shaken for an hour and half on a vibrator (Syntron Paper Jogger Type PJ 4) The suspension was kept in refrigerator overnight. The next day it was shaken again for 1 hour It was then centrifuged at 14,500 RPM (about 27,000 g.) for 4 hours. The supernatant was filtered through an ultra-fine ground glass filter The filtrat which was clear straw-colored fluid was lyophilized, stored in refrigerator and reconstituted with physiologic saline solution just before use.

The sediment was washed 5 times with water, lyophilized, stored for future use in refrigerator and resuspended at least 24 hours before use in physiologic saline solution containing 0.01 % Merthiolate.

One mg. of ell walls was prepared from approximately 230,000,000 cells, one mg. of the soluble extract from about 100,000,000 cells.

Animals. — Male white Swiss mice weighing 20—25 gm. were housed 10 to metal cage, fed commercial pellets (supplied by Hemlock Hollow Farm, Wayne, N. J.) and given water *ad libitum.* Male guinea pigs weighing 180—220 gm. were used in hypersensitivity experiments.

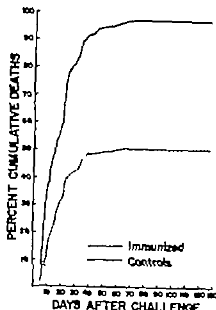


Fig 1 Cumulative death rates of mice immunized sc with cell walls of *Candida albicans* and challenged with 1 000,000 yeast cells of *C. albicans* intravenously (95 mice) Controls (100 mice) received 0.2 cc. of saline solution instead of the vaccine.

(b) Specific Immunization with Cell Walls of Candida albicans

In preliminary experiments either 15 9 or 6 injections of varying amounts of cell walls were administered subcutaneously to mice within 19 days. Eighteen days after the last injection mice were challenged intravenously with 1 000,000 cells of *Candida albicans* The individual immunizing injections contained either 0 1 mg 1 mg., 5 mg 10 mg 25 mg 50 mg 75 mg 100 mg or 200 mg of cell wall material per injection. While no appreciable effect was noted with 0 1 mg or 1 mg the life of mice receiving 5 mg 10 mg 25 mg 50 mg 75 mg or 100 mg was considerably prolonged. Most mice receiving 200 mg died in the course of immunization.

In another experiment 100 mic were injected 8 times with 5 mg of the cell wall material in 0.2 cc. of phy siologic sal ne sol tion 38 35 33 31 28, 26, 24 and 21 days prior to intra enous challenge with 1,000 000 cells of *Candid albicans* F e of the 100 mice died between the first immunizing injection and challenge. The remaining 95 mice were observed daily for 126 days after challenge. The dead animals were autopsied. A control group of 100 mice received injections of 0.2 cc. of saline on the days on which other animals were immunized with the cell wall material. Ninety-seven of 100 control mice were dead at the end of the experiment while 48 (50.5 %) of 95 immunized animals died. Death rates in this experiment are illustrated in Figure 1

On autopsies of both immunized and nonimmunized animals lesions in various organs, mainly the kidneys, were noted. Lesions in most immunized animals appeared less extensive than those of the controls.

Ten mice similarly pretreated with cell walls of *Candida albicans* received an intraperitoneal injection of 0.2 cc. of a 1 20 dilution of a 24 hour broth culture of *Salmonella enteritidis* 21 days after the last vaccine injection. These mice died at about the same time as ten controls which received saline solution instead of the cell wall vaccine.

The mice given 5 mg of cell wall material 8 times developed indurations at the sites of the subcutaneous injections most of which disappeared entirely after several weeks. Mice recei ing higher doses of the cell walls occasionally developed necrotic lesions in the subcutaneous tissue at times with ulcerations of the skin.

(c) Failure to Confer Specific Immunity with Soluble Extract of Candida albicans

Two experiments were performed to determine wl ether protection against a hallenge with *Candid alb cans* is possible by repeated subcutaneous njections f the soluble extract of the fungus th last one of whi h is given mor tha tw weeks befor hal lenge

In one experiment total 1 70 mic wer given either 9 18 or 32 subcutaneous n-jection of the soluble extra t of *C albic* within 20 days. The doses ranged from 1 t 100 mg f th ext act per injection Eight een days fter the la t injection the mic were challenged i tra enously with either

1,200,000 or 12,000,000 cells. There was no difference between the survival times of immunized and nonimmunized mice.

In the second experiment a total of 80 mice were given either 9, 19 or 33 subcutaneous injections of the soluble extract of *Candida albicans* within 21 days. The doses again ranged from 1 to 100 mg. Eighteen days after the last injection the animals were challenged intravenously with 1,000,000 cells. There was no difference between the survival times of immunized and nonimmunized animals. Similarly as in the preceding experiment, mice receiving higher doses of the extract developed extensive alopecia in the course of the treatment.

(d) Changes in Nonspecific Resistance to *Candida albicans* Induced by Soluble Extract of *C. albicans* and Endotoxin of *Escherichia coli*

In an exploratory experiment two mice were given 33 intraperitoneal injections of 150 mg each of the soluble extract of *Candida albicans* within 21 days. Fourteen days later they were challenged intravenously with 6,000,000 living cells of *C. albicans*. Their survival time was 6 and 7 days, respectively, as compared to 10 controls injected with saline in which the average survival time was 3.2 days.

This result pointed to the possibility that some protection could be afforded the animals when using the intraperitoneal route of inoculation and when shortening the time between the last injection and challenge. It appeared possible that the results of this experiment may be interpreted as an increase in nonspecific resistance. In bacterial infections the effects of nonspecific resistance are of much more limited duration than those of specific immunity and intraperitoneal injections are much more effective in inducing it than subcutaneous ones.

My previous experiments showed that the soluble extract produced toxic symptoms and hematological changes similar to those caused by endotoxins of gram-negative bacteria. The experiment just described created suspicion that this endotoxin-like substance

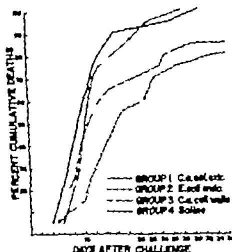


Fig. 2. Effect of an ip injection of 200 mg of soluble extract of *Candida albicans* (Group 1), 35 µg. of endotoxin of *Escherichia coli* (Group 2) and 5 mg. of cell walls of *C. albicans* (Group 3) on death rates of mice challenged iv with 6,000,000 yeast cells of *C. albicans* 5 days after pretreatment. Controls (Group 4) were pretreated with 0.5 cc. of saline solution ip. Each group 20 mice.

may share still another property with gram-negative endotoxins, namely their ability to modify nonspecific resistance to infections.

An experiment was set up, therefore, to test this possibility. Twenty mice were injected intraperitoneally with 200 mg. of the soluble extract of *Candida albicans*. 20 mice received an intraperitoneal injection of 35 µg. of the endotoxin of *Escherichia coli*. 20 animals were given 5 mg. of cell walls of *C. albicans* and 20 mice received 0.5 cc. of physiologic saline solution by the same route. Five days later all animals were given an intravenous injection of 6,000,000 viable yeast cells of *C. albicans*. Figure 2 shows that the survival times of mice pretreated with either the *Candida* extract or the endotoxin of *Escherichia coli* were prolonged. There was only a very slight prolongation of life in mice pretreated with cell walls of *C. albicans*.

In an experiment in which 20 mice were injected with 275 mg. of the soluble extract

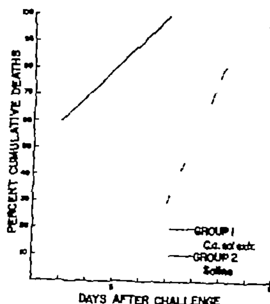


Fig 3 Effect of an ip injection of 275 mg of soluble extract of *Candida albicans* (Group 1) on death rates of mice challenged i with 12 000 000 *yeast cells of C albicans 5 days later* Controls (Group 2) were pretreated with 0.5 cc. of saline solution p. Each group, 20 mice.

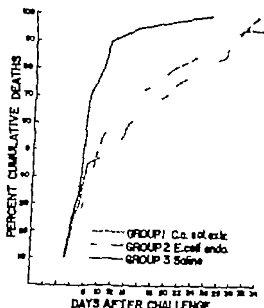


Fig 5 Effect of an ip injection of 200 mg of soluble extract of *Candida albicans* (Group 1), and of 35 µg of *Escherichia coli* endotoxin (Group 2) on death rates of mice challenged with 6,000,000 yeast cells f C. albicans 14 days later Controls (Group 3) were pretreated with 0.5 cc of salin solution ip. Each group, 20 mice

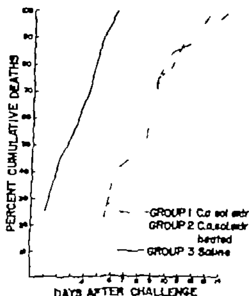


Fig 4 Effect of a *ip injection f 275 mg of* soluble extract of *Candida albicans* (Group 1) and of 275 mg of the same extract exposed to 60° C for 1 hour (Group 2) on death rates of mice challenged *with 12,000 000* yeast cells of *C. albicans 8 day* later Controls (Group 3) were pretreated with 0.5 cc of saline solution *ip. Each* group, 20 mice

of *C lbicans* intraperitoneally 5 days before a challenge with 12,000 000 viable cells of *C albicans* the survival time was considerably prolonged as compared with controls injected with saline instead of the extract (*Figure 3*)

A similar result was observed in mice in hich the interval between the pretreatment and challenge was 8 days (Figure 4) In this experiment 20 mice were injected with 275 mg of the soluble extract of *C albicans* 20 mice w th the same extract heated for 1 hour at 60 C and 20 mice w th saline solution. There was no essential difference in mortality rates between mice t eated with the heated and unheated extra t The effect *of the Candida* extract *appeared* latn ly more pronoun ed in mice *challenged with* 12 000 000 c lls (*Figure 3* and *4*) *than in those challenged w th 6,000 000 cells* (*Figur 5*) Figure 3 indicates that all of the control mice were dead 5 days after challenge th day on which the first de th occurred in th treated group. Some effect f both the *Can*

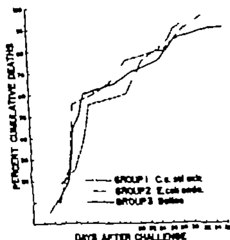


Fig. 6. Effect of an ip injection of 200 mg of soluble extract of *Candida albicans* (Group 1) and of 35 μg of *Escherichia coli* endotoxin (Group 2) on death rates of mice challenged with 6,000,000 yeast cells of *C. albicans* 30 day later. Controls (Group 3) were pretreated with 0.5 cc. of saline solution ip. Each group 20 mice.

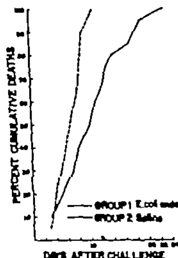


Fig. 8. Effect of an ip injection of 35 μg of *Escherichia coli* endotoxin (Group 1) administered 3 days after an iv injection of 6,000,000 yeast cells of *Candida albicans* on death rates of mice. Controls (Group 2) received 0.5 cc. of saline solution ip instead of endotoxin. Each group, 20 mice.

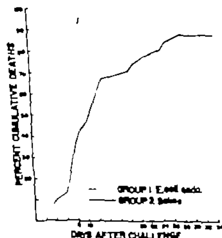


Fig. 7. Effect of an ip injection of 35 μg of *Escherichia coli* endotoxin (Group 1. 20 mice) administered 3 hours after an iv injection of 6,000,000 yeast cells of *Candida albicans* on death rates of mice. Controls (Group 2. 30 mice) received 0.5 cc. of saline solution ip instead of endotoxin.

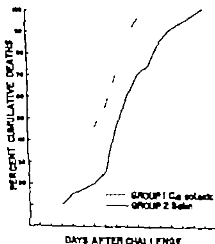


Fig. 9. Effect of an ip injection of 200 mg of soluble extract of *Candida albicans* (Group 1) administered 3 hours after an iv injection of 6,000,000 yeast cells of *C. albicans* on death rates of mice. Controls (Group 2) received 0.5 cc. of saline solution ip instead of the extract. Each group, 20 mice.

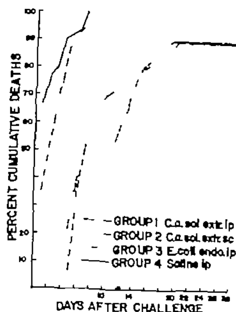


Fig 10 Effect of 2 ip (Group 1 20 mice) or sc (Group 2 20 mice) injections of sol ble extract of *Candid albicans* given on first (150 mg.) and second (200 mg) day f experiment, and of 2 p jections of *Escherichia col* endotoxin (Group 3 20 mice) given t the same times (25 μg. each time) on p challenge with 0 15 cc of 1 10 d'lution of a broth culture f *Salmonella enteritid* given on the fourth day of the experiment. Controls (Group 4 30 mice) were pretreated w th njections f 0.5 cc of sali e solution ip

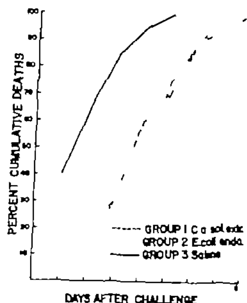


Fig 11 Effect f an ip injection of 200 mg soluble extract of *Candida lbicans* (Group 1) and of 35 μg f *Escherichia col* endotoxin (Group 2) on death rates of m ce challenged with 0.2 cc. of a 1 20 dilution of a broth culture f *Salmonella enteritidis* 5 day fter pretreatment Controls (Group 3) were pretreated with 0.5 cc of saline sol tion ip. Each group, 20 mice

dida extract and *E coli* endotoxin was still p esent when the interval between pretreat ment and challenge was 14 days (Figure 5) but no effect on the survival times could be detected when the interval was 30 days (Figure 6)

The survi al tim of mice was shortened when *E coli* endotoxin was injected 3 hours (Figure 7) or 3 days (Figu e 8) after the *Ca dida* infection. A similar result was obtained hen the soluble extract of *C albicans* was injected 3 hours after the infect on with *C alb ca s* (Figure 9)

(e) *Changes in Nonspecific Resistance t Salmonella enteritidis Induced by Soluble Extract of Candida albicans and Endotoxin of Escherichia coli*

Twenty mice wer njected intrape toneally with 150 mg of the sol ble extract of *Candida albicans* on the first da of the experiment. On the second da 200 mg f the sam material w s injected intraperitoneally Anoth group of 20 animals was injected at th same tim subcutaneously with the same amount f the *Candid* extract A third group of 20 animals rec ved 2 injec tions of 25 μg ea h f th endot xin of *E col* intraperitoneally inj d. A fourth group of 30 a imal served ontr ls and rece ed ph iological salin solu ion traperitoneal- l Mi all 4 gro ps w r h llenged w th an intraperitone l ject on of 0 2 of 1 20 dil tion of a 24 hour broth ul t of *Salm ll t d* on th fou th da of th e xperiment An mal pr reated i traperitoneally with th C d d t t or w th *E c li* endotoxin urvived on id- rabl longer than the controls wh h dev l ped an ut i fection and two th d f th em (20 f 30) died w th n 4 hours The la t mouse of th ontrol group died 7 da

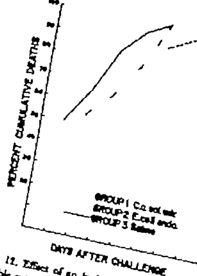


Fig. 12. Effect of an ip injection of 200 mg. of soluble extract of *Candida albicans* (Group 1) and of 35 µg. of endotoxin of *Escherichia coli* (Group 2) on death rates of mice challenged with 0.2 cc. of 1 : 20 dilution of broth culture of *Salmonella enteritidis* 14 days after pretreatment. Controls (Group 3) were pretreated with 0.5 cc. of saline solution ip. Each group, 20 mice.

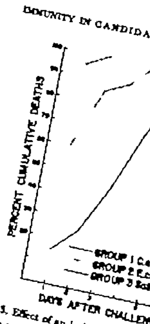


Fig. 13. Effect of an ip injection of 200 soluble extract of *Candida albicans* (Grou of 35 µg. of *Escherichia coli* endotoxin (G on death rates of mice challenged with of 1 : 40 dilution of broth culture of *Salmonella enteritidis* 2 hours after the pretrea Controls (Group 3) were pretreated ith of saline solution ip. Each group, 20 mice

after inoculation. One third of mice pretreated with the *Candida* extract intraperitoneally were still alive after 13 day and one third of mice pretreated with *E. coli* endotoxin were alive 9 days after challenge. Only slight protection was observed among mice pretreated subcutaneously with the *Candida* extract. Figure 10 illustrates this experiment.

In another experiment (Figure 11) one group of 20 mice was given a single intraperitoneal injection of 200 mg. of the soluble extract of *Candida albicans*, the second group of 20 mice received a single intraperitoneal injection of 35 µg. of the endotoxin of *E. coli* and the third group of 20 control mice received saline solution. Five days after the pretreatment mice in all 3 groups were challenged with an intraperitoneal injection of 0.2 cc. of a 1 : 20 dilution of broth culture of *S. enteritidis*. Mice receiving *Candida* extract or *E. coli* endotoxin survived longer than the controls. Their survival time was not prolonged as much as that of mice which received 2 injections of either the Candida extract or *E. coli* endotoxin in the preceding experiment. When the interval between pretreatment and challenge was 14 day the prolongation of life was only slight (Figure 12).

Enhancement of *Salmonella* infection was demonstrated when *Salmonella enteritidis* was injected 2 hours after either the *Candida* extract or the endotoxin of *E. coli* (Figure 13).

(f) Sensitization with Soluble Extract of *Candida albicans*

Twelve guinea pigs were injected intraperitoneally with various amounts of the soluble extract of *Candida albicans*. Eighteen days later varying amounts of the same material were injected intracardially to 6 of them, the remaining 6 guinea pigs were given the shocking dose of the extract intraperitoneal.

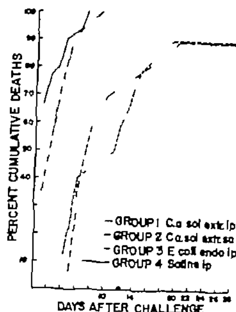


Fig 10 Effect of 2 ip (Group 1 20 m ce) or sc (Group 2 20 mice) injections of soluble extract f *Candida albicans* given on first (150 mg) and second (200 mg) day f experiment, and of 2 ip injections of *Escherichia coli* endotoxin (Group 3 20 mice) given t the same times (25 µg each time) on p challenge with 0 15 cc of a 1 10 dilution of a broth cultur of *Salmonella enteritidis* given on the fourth day of the experiment. Controls (Group 4 30 mice) were pretreated with i jections of 0 5 cc of saline solution ip

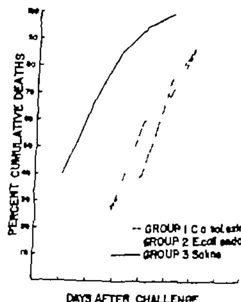


Fig 11 Effect f an ip i jection of 200 mg o sol ble extract of *Candida albicans* (Group 1) and of 35 µg of *Escherichia coli* endotoxin (Group 2) on death rates of mice challenged with 0.2 cc. of a 1 20 dilution f a broth culture of *Salmonella enteritidis* 5 days after pretreatment. Controls (Group 3) were pretreated with 0.5 cc of saline solution ip. Each group 20 mice

dida extract and *E coli* endotoxin was still present when the interval between pretreatment and challenge was 14 days (Figure 5) but no effect on the survival times could be detected when the interval was 30 days (Figure 6)

The survival time of mice was shortened when *E c li* endotoxin was injected 3 hours (F gure 7) or 3 days (Figure 8) after the *C ndid* infection A si nilar result was obtained when the solubl extract of *C albi a* wa injected 3 hours aft r the nfect on with *C albicans* (Figure 9)

(c) Changes in Nonspecific Resistance to Salmonella enteritidis Induced by Soluble Extract of Candida albicans and Endotoxin of Escherichia coli

Twenty m c wer njected ntraper toneally with 150 mg of the solubl extra t of *Candida albicans* on the first day of the experiment On the second day 200 mg of the same material was injec d intraperitoneally Another group of 20 animals wa injected at the same t me subcutaneously with the same amount of the *Candida* extract A th rd group of 20 anim ls eceived 2 injections of 25 g ea h of the endotoxin of *E. c l* intraperitoneally instead A fourth group of 30 animal erved s ontrol and recei ed ph ologi al saline solution ntraper toneally All in all 4 grou ps were hallenged with an i traperitoneal injection f 0 2 c of 1 20 d l tion f 24 hou brot l tu e of *Salm lla nt t d* on the fourth day of th experiment Animal pret ted ntr pe itoneally w th th *C d d* tra t w th *E c l* endotoxin survi ed on id bl longer than the control wh ch dev l oped t infection and in th rd f them (20 of 30) d ed w thin 24 ho rs The l st mouse of the control group died 7 d y

(3) REVIEW OF LITERATURE

A detailed critical review of past experimental work may be desirable for better understanding of the successes and pit falls of immunization against moniliasis, and of certain aspects of the pathogenicity of *Candida*. It is believed that most of these vaccination trials may be satisfactorily interpreted on the basis of the experimental findings reported in this paper which clarify some aspects of nonspecific host resistance, hyper sensitivity and localization of various immunologically active materials in distinct cell constituents of *Candida*. The limited past experimental findings in these fields have not been taken into account or correlated in planning immunization experiments in which the choice dosage timing and route of administration of both the antigens and the materials used for challenge are factors of utmost importance.

Comprehensive reviews of the subject of immunization in moniliasis are not available. Fischl (33) discussed earlier immunization trials while only three recent protection experiments were mentioned in the review of Levine (73). Reports on the effect of various microorganisms or their fractions on nonspecific resistance to *Candida* are scatter ed in the literature. No systematic at tempt has been made to explain the phenomena of enhancement or depression of resistance in such situations, an ttempt which has been undertaken for similar occurrences in bacterial infections (6, 8, 9, 62, 114 134)

A search of the earlier literature on moniliasis appeared generally more re warding than a review of more recent articles on the subject. This applied not only to the experimental work but also to clinical and pathological descriptions. One of the best articles in the latter category was written by Valleix (142) as early as 1838, one year before the fungus of thrush was discovered. His work demonstrates that generalized moniliasis, a condition in which management based on immunological principles may be of value, had been a well known clinical entity more than a century before the discovery of antibiotics. The importance of disseminated moniliasis should not be underestimated today at a time in which enhancement of *Candid* infections by antibiotics and other modern drugs may play an important role in some parts of the world, while sanitary conditions probably not unlike those of Paris in 1838 may contribute to its development in other less developed regions.

Most of the important experimental findings concerning the immunology of *Candida* have been made more than quarter of a century ago. Concetti (14) based on a small experiment, postulated in 1900 that protection against moniliasis was possible by injections of cell walls of *Candida*. del Pont (16) has shown

Table 1 Attempts to sensitize guinea pigs with soluble extract of Candida albicans and with its cell walls

Guinea pig No.	Sensitizing injection	mg.	Challenging injection	mg	Outcome
1	Sol. extr ip	5	Sol. extr ic[1]	50	2
2	Sol. extr ip	10	Sol. extr ic	100	Died
3	Sol. extr ip	10	Sol. extr ic	100	3
4	Sol. extr ip	50	Sol extr ic	200	3
5	Sol. extr ip	100	Sol. extr ic	200	2
6	Sol. extr ip	100	Sol. extr ic	500	Died
7	Sol. extr ip	5	Sol. extr ip	50	2
8	Sol extr ip	10	Sol. extr ip	100	3
9	Sol. extr ip	10	Sol. extr ip	100	2
10	Sol. extr ip	50	Sol. extr ip	200	No reaction
11	Sol extr ip	100	Sol extr ip	200	3
12	Sol. extr ip	100	Sol. extr ip	500	2
13	Cell walls ip	1	Cell walls ip	10	No reaction
14	Cell walls ip	5	Cell walls ip	25	No reaction
15	Cell walls ip	10	Cell walls ip	25	No reaction
16	Cell walls ip	10	Cell walls ip	25	No reaction
17	Cell walls ip	10	Cell walls ip	50	No reaction
18	Cell walls ip	25	Cell walls ip	50	No reaction
19	None		Sol. extr ic	100	No reaction
20	None		Sol. extr ic	100	No reaction
21	None		Cell walls ip	25	No reaction
22	None		Cell walls ip	25	No reaction

The challenging injection was given 18 days after the sensitizing dose.
ic = intracardially 2 = moderate, 3 = severe anaphylactic shock.

ly All six guinea pigs given the intracardial injection developed symptoms of a moderate to severe anaphylactic shock within 1 to 3 minutes. The symptoms included ruffled hair restlessness cough cyanosis, rubbing of nose, marked respiratory distress, convulsions, weakness. Two of the animals died within 5 minutes, the remaining 4 recovered.

Five of the six guinea pigs which received the challenging dose intraperitoneally developed symptoms of a moderate to severe anaphylactic shock but all of them recovered.

Six guinea pigs were injected intraperitoneally with various amounts of cell walls of *Candida albicans* On the eighteenth day of the experiment they received a challenging dose of the same material intraperitoneally None of the animals developed signs of anaphylactic shock.

Two control animals, not previously sensitized, received 100 mg of the soluble extract of *Candida albicans* intracardially Two other nonsensitized guinea pigs were given an intraperitoneal injection of 25 mg of cell walls of *C albicans* None of these four animals developed any symptoms of anaphylactic shock. These experiments are summarized in Table 1

(3) REVIEW OF LITERATURE

A detailed critical review of past experimental work may be desirable for better understanding of the successes and pitfalls of immunization against moniliasis, and of certain aspects of the pathogenicity of *Candida*. It is believed that most of these vaccination trials may be satisfactorily interpreted on the basis of the experimental findings reported in this paper which clarify some aspects of nonspecific host resistance, hypersensitivity and localization of various immunologically active materials in distinct cell constituents of *Candida*. The limited past experimental findings in these fields have not been taken into account or correlated in planning immunization experiments in which the choice, dosage, timing and route of administration of both the antigens and the materials used for challenge are factors of utmost importance.

Comprehensive reviews of the subject of immunization in moniliasis are not available. Fischl (33) discussed earlier immunization trials while only three recent protection experiments were mentioned in the review of Levine (75). Reports on the effect of various microorganisms or their fractions on nonspecific resistance to *Candida* are scattered in the literature. No systematic attempt has been made to explain the phenomena of enhancement or depression of resistance in such situations, an attempt which has been undertaken for similar occurrences in bacterial infections (6, 8, 9 62, 114 134).

A search of the earlier literature on moniliasis appeared generally more rewarding than a review of more recent articles on the subject. This applied not only to the experimental work but also to clinical and pathological descriptions. One of the best articles in the latter category was written by Valleix (142) as early as 1838, one year before the fungus of thrush was discovered. His work demonstrates that generalized moniliasis, a condition in which management based on immunological principles may be of value, had been a well known clinical entity more than a century before the discovery of antibiotics. The importance of disseminated moniliasis should not be underestimated today at a time in which enhancement of *Candida* infections by antibiotics and other modern drugs may play an important role in some parts of the world, while sanitary conditions probably not unlike those of Paris in 1838 may contribute to its development in other less developed regions.

Most of the important experimental findings concerning the immunology of *Candida* have been made more than a quarter of a century ago. Concetti (14) based on small experiment, postulated in 1900 that protection against moniliasis was possible by injections of cell walls of *Candida*. del Pont (16) has shown

in 1904 the feasibility of passive immunization. Kurotchkin and Lim (68) concluded in 1933 that sensitization was one of the possible mechanisms for generalization of *Candida* infections. Ninni and Fittipaldi (96) have noticed in 1934 increased susceptibility to *Candida* infection in animals given repeated frequent small doses of the fungus, a phenomenon apparently based on the resistance-influencing effect of the endotoxin like substance of *Candida*. No attemps have been made in the intervening period to correlate these findings or to utilize them for the management of moniliasis or any other fungus disease by immunological means.

The review of literature is divided into three sections, viz. nonspecific host resistance, allergy and its relation to immunity and specific immunity Each of the reviewed experiments was classified under the heading which appeared to have played the most important role in inducing the described changes. Since several factors may have been responsible for the changes in some experiments this classification may only be an arbitrary one.

(A) Nonspecific Host Resistance

The literature on the role of the fungi of the genus *Candida in nonspecific host* resistance is reviewed here since its knowledge *is helpful in evaluating* the experimental work on specific immunity *which will* be discussed later in this paper Much influence on the ultimate *outcome* of immunization appears to be exerted by the endotoxin like substance of the soluble fraction which, depending on timing either enhances or depresses the resistance to *Candida* and may thus enhance or nullify the effect of the protective antigen which, as was shown by own experiments, apparently resides in the cell walls of *Candida*.

Candida has been used either as an organism to which nonspecific resistance was induced, or an agent or source of substances for the enhancement or depression of resistance to other microorganisms.

Studies in which *Candida* was used in an animal both as a modifying agent and an infecting organism, and in which there is a relatively short interval between pretreatment and challenge, are included here rather than in the section on specific immunization. It is probable that the change in the course of the infection in such experiments is not based on mechanisms of specific immunity

The experiments dealing with nonspecific resistance may be divided into two groups.

1) The first group includes experiments in which the agent used for modification of the infection was either a dead microorganism, an extract therefrom or a substance unrelated *to* a microorganism. In these experiments the agent may *be accurately measured* and certain variables inherent in the use of a *living and multiplying* agent are eliminated This group may be subdivided into the following sections

(a) Experiments in which enhancement or depression of nonspecific resistance against a *Candida* infection was induced using various dead microorganisms or their products or substances not derived from microorganisms.

(b) Studies in which dead *Candida* or its products were used to enhance or depress resistance against a *Candida* infection.

(c) Experiments in which dead fungi of the genus *Candida* or their products were used to induce enhancement or depression of resistance against other microorganisms in animals.

(ii) In the second group are included experiments in which living microorganisms were administered in two or more injections. The organism injected in one or all of them was *Candida*. Injections of two different organisms were given either simultaneously or at varying intervals between each other.

Because of varying rates of multiplication, production and liberation of substances effective in modifying infections different microorganisms at different dosage levels and routes of administration may cause response which, unlike in the experiments of the first group, cannot be easily predicted. In some instances the substance from the first injection, if more potent, may nullify the effect of that from the second injection, or vice versa. The size of the inoculum determining among other factors the rate of spread of the microorganism in the animal, may conceivably influence the time of maximum liberation of the endotoxin-like substance deciding thus whether the infection will be enhanced or depressed. This second group may be subdivided into the following sections:

(a) Studies of the effects caused by injections of living *Candida* and other living microorganisms in animals. Changes in the course of such mixed infections may be interpreted as enhancement or depression of nonspecific host resistance induced by endotoxin-like substances from either *Candida* or the bacterial organism in question, or the effect of antibacterial or antifungal substances which these organisms may produce.

(b) Studies of the effects of living *Candida* cells on the resistance to a *Candida* infection.

(i) (a) *Effect of Dead Organisms, their Products or Substances not Derived from Microorganisms on Nonspecific Resistance to Candida*

Products of bacterial organisms and fungi, as well as sterile milk, were used to modify resistance to *Candida* infections.

Gale and Sandoval (37) injected various concentrations of heat-killed cells of *Escherichia coli* or 1.0 to 100 µg of *E. coli* endotoxin 30 minutes after an intravenous injection of *Candida albicans* into mice and found that their survival time was shortened as compared to control mice injected with *Candida* alone.

These experiments demonstrate that, similarly as in bacterial infections, endotoxins stimulate the growth of fungi when injected shortly after a fungal challenge.

Hasenclever and Mitchell (41) injected mice intraperitoneally with 30 µg of the endotoxin of *Salmonella enteritidis* 1 to 14 days before an intravenous challenge with 10^7 to 2×10^7 cells of *Candida albicans*. The survival times of controls were less than 24 hours. The

lives of mice pretreated either 1 or 6 days before challenge were prolonged while no essential prolongation of survival times was noted in mice treated 3 10 or 14 days before challenge. Mice given 20 μg of the endotoxin of *Salmonella typhosa* or *S enteritidis* 6 days before and 30 μg one day before challenge lived somewhat longer than those receiving a single injection of endotoxin. Animals given 20 μg of *S enteritidis* endotoxin 7 days, and 30 μg 6 days and 1 day before challenge survived the longest. Mice treated with 10^7 viable cells of *C albicans* and 30 μg of *S enteritidis* endotoxin intraperitoneally 6 days before challenge lived somewhat longer than those given the endotoxin alone. The same was true of mice receiving 10^7 viable cells of *C albicans* 6 days and 30 μg of *S enteritidis* endotoxin 1 day before challenge. Prolongation of survival times after pretreatment with endotoxin was observed in mice 6 or 12 weeks old but 9-week-old mice failed to respond.

Partial protection of mice pretreated with endotoxin 1 or 6 days before challenge is in agreement with own experiments in which this effect was observed when endotoxin of *Escherichia coli* or the soluble extract of *C albicans* was injected 5 8 or 14 days before challenge with *C albicans* (Figures 2, 3 4 and 5) Hasenclever and Mitchell did not see however any effect when the interval between pretreatment and challenge was 3 10 or 14 days.

Other experiments of the same authors show that there was no essential change in the survival times of mice given 1 mg of nonviable *Mycobacterium butyricum* intraperitoneally or those receiving 0.1 ml. of oil by the same route 6 days before challenge The intraperitoneal administration of the complete Freund's adjuvant containing 1 mg of *M butyricum* in 0.1 ml of oil prolonged the survival times of the mice somewhat.

Scherr (129) injected mice intraperitoneally with 10^9 formalin treated cells of *Saccharomyces cerevisiae* every other day except for a 5-day rest period after the sixth day The injections were started 24 hours after an intraperitoneal administration of 5×10^6 cells of *Candida albicans* When the mice were sacrificed 20 days after the infection with *Candida* it was found that the degree of dissemination of moniliasis was much more pronounced in animals injected with formalin-treated *Saccharomyces* than in untreated controls which received only *Candida*

In another experiment mice received ten daily intraperitoneal injections of a supernatant remaining after grinding of an aqueous suspension of *Saccharomyces* with sand in a mortar The supernatant was not concentrated This treatment was started 24 hours after an intraperitoneal injection of one half the amount of the *Candida* suspension used in the preceding experiment Treatment with this material had no effect on the dissemination of moniliasis.

Another group of mice received 5×10^6 cells of *C albicans* interaperitoneally Twenty four hours later injections of concentrated culture filtrates of *S cerevisiae* were started and repeated every other day for a total of 10 injections When the mice were sacrified 20 days after the injection of *Candida* it was found that the degree of dis-

germination of monilias was about the same as in untreated controls which received only *Candida*.

The experiments with dead cells of *S cerevisiae* given repeatedly to mice after they had been infected with *Candida* demonstrate the same dependence of the effect on timing which was seen in the work of Gale and Sandoval (36) and in own experiments (Figures 7 and 8) The finding that small amounts of an extract prepared by grinding of *S cerevisiae* which was not concentrated did not have any effect on the resistance to *Candid* may have been caused by the fact that too little of the active principle was injected. It is not surprising that culture filtrates of *S. cerevisiae* were ineffective. If an analogy exists between the location of the resistance-influencing substance of *Candid* and *S cerevisiae* the active principle would be expected to be present in a soluble cell fraction of *S cerevisiae*

Whitney Parfentjev and Whitney (149) injected mice intraperitoneally with single dose of malucidin, a yeast extract, 24 or 48 hours before an intra peritoneal injection of 100,000,000 cells of *Candida albicans*. Five hours before the challenge dose of *Candid* the mice received 2 mg of aureomycin intra peritoneally Without this antibiotic pretreatment the fungal challenge was not lethal. Pretreatment with malucidin of aureomycin-enhanced infection protected many mice during the acute phase of moniliasis. Forty-two out of 100 mice given various batches and dosage levels of malucidin were still living after 12 days while all control mice which were given aureomycin and *Candid* died.

This protection against *Candida* may also be due to the same mechanism. It is apparently not based on any antibiotic effect since no inhibition of growth was noted when *C albicans* was incubated with malucidin in vitro. This and the preceding experiment of Scherr (129) point to the possibility that *Saccharomyces cerevisiae* contains a substance similar to that present in the soluble fraction of *Candida* or in gram-negative organisms, which is effective in influenc ing nonspecific resistance. It is perhaps interesting that such substances may also be present in nonpathogenic fungal organisms.

Hasenclever and Mitchell (40 41) injected mice intraperitoneally with 1 mg of formalin-killed mechanically disintegrated spherules of *Coccidioides immitis* six days before they challenged them intravenously with 10^7 cells of *Candida albicans* The hf of pretreated mice was somewhat prolonged as compared with mice which received *Candida* challenge only

This experiment points to the possibility that *Coccidioides immitis* may contain an endotoxin-like substance, similar to that of *Candida*, which is capable of influencing nonspecific resistance.

Fischer and Horbach (31) pretreated 29 mice with 0.5 cc. of sterile milk intra peritoneally 24 hours before they challenged them by the same route with 0.2 cc. of a 4 % suspension of *Candida albicans*. Only six of the pretreated mice died in 3 weeks while there were 15 deaths among 30 controls which received the challenge dose only

In this experiment milk appeared to have protective effect. Such a property

lives of mice pretreated either 1 or 6 days before challenge were prolonged while no essential prolongation of survival times was noted in mice treated 3 10 or 14 days before challenge. Mice given 20 μg of the endotoxin of *Salmonella typhosa* or *S enteritidis* 6 days before and 30 μg one day before challenge lived somewhat longer than those receiving a single injection of endotoxin. Animals given 20 μg of *S enteritidis* endotoxin 7 days, and 30 μg 6 days and 1 day before challenge survived the longest. Mice treated with 10^7 viable cells of *C albicans* and 30 μg of *S enteritidis* endotoxin intraperitoneally 6 days before challenge lived somewhat longer than those given the endotoxin alone. The same was true of mice receiving 10^7 viable cells of *C albicans* 6 days and 30 μg of *S enteritidis* endotoxin 1 day before challenge. Prolongation of survival times after pretreatment with endotoxin was observed in mice 6 or 12 weeks old, but 9-week-old mice failed to respond

Partial protection of mice pretreated with endotoxin 1 or 6 days before challenge is in agreement with own experiments in which this effect was observed when endotoxin of *Escherichia coli* or the soluble extract of *C albicans* was injected 5 8 or 14 days before challenge with *C albicans* (Figures 2, 3 4 and 5) Hasenclever and Mitchell did not see, however any effect when the interval between pretreatment and challenge was 3 10 or 14 days.

Other experiments of the same authors show that there was no essential change in the survival times of mice given 1 mg of nonviable *Mycobacterium butyricum* intraperitoneally or those receiving 0 1 ml. of oil by the same route 6 days before challenge. The intraperitoneal administration of the complete Freund's adjuvant containing 1 mg of *M butyricum* in 0.1 ml. of oil prolonged the survival times of the mice somewhat

Scherr (129) injected mice intraperitoneally with 10 formalin-treated cells of *Saccharomyces cerevisiae* every other day except for a 5-day rest period after the sixth day The injections were started 24 hours after an intraperitoneal administration of 5×10^5 cells of *Candida albicans* When the mice were sacrificed 20 days after the infection with *Candida* it was found that the degree of dissemination of moniliasis was much more pronounced in animals injected with formalin-treated *Saccharomyces* than in untreated controls which received only *Candida*.

In another experiment mice received ten daily intraperitoneal injections of a supernatant remaining after grinding of an aqueous suspension of *Saccharomyces* with sand in a mortar The supernatant was not concentrated This treatment was started 24 hours after an intraperitoneal injection of one half the amount of the *Candida* suspension used in the preceding experiment. Treatment with this material had no effect on the dissemination of moniliasis.

Another group of mice received 5 × 10 cells of *C albicans* interaperitoneally Twenty four hours later injections of concentrated culture filtrates of *S cerevisiae* were started and repeated every other day for a total of 10 injections. When the mice were sacrified 20 days after the injection of *Candida* it was found that the degree of dis-

The death rate of pretreated mice was somewhat lower than that of the controls. If any significance may be attached to this experiment, the difference may be explained by the presence of the endotoxin-like substance in the first injection. However possibly because of the short time interval between the first and second injection, the protection was not too pronounced.

Hasenclever and Mitchell (40) injected mice intraperitoneally or intravenously with 10^7 or 5×10 heat killed cells of *Candida albicans* and challenged them 6 days later intravenously with 10^7 viable cells of the same fungus. There was not much difference in the survival time between pretreated mice and controls except for a group pretreated intraperitoneally with the higher dose, 5×10^7 dead cells, in which the survival time was somewhat prolonged.

This experiment may also be interpreted on the basis of the protective effect of the endotoxin-like substance contained in the dead cells of *C albicans* when administered several days before challenge The number of cells in the pretreatment dose may have been somewhat low which may explain some effect only with 5×10^7 heat killed cells. Although great individual variations in the response of mice may be expected from one experiment to another it should be noted that Scherr (129) used about eight doses of 10^8 dead cells each, and Fischer and Horbach (31) injected 10^9 dead cells once I own experiments 200 mg of soluble extract which was found effective for the enhancement or depression of nonspecific resistance was prepared from about 20,000,000,000 cells.

Isenberg, Allerhand and Berkman (54) injected mice intravenously with 0.2 cc. of an aqueous fraction isolated by ethanol-ethyl ether or phenol extraction of intact cells of *Candida albicans*. Six days later they challenged them intravenously with 10 million cells of the homologous strain The pretreated mice were protected while the controls died. The number of experimental animals or the length of the observation period were not given.

The experiments in this group demonstrate the resistance-influencing effects of formalin-treated or heat-killed cells of *Candid* or a *Candid* extract on *Candida* infections. Repeated injections of dead cells given after infection with *Candida albicans* enhance the growth of the fungus. Dead cells of *C albicans* or its extract given several days before challenge have a protective effect. These findings are confirmed by own experiments in which a soluble extract of *C albicans* was used (Figures 2, 3 4 5 and 9) The resistance-influencing activities of the endotoxin-like substance of *Candida* are pparently not destroyed after exposure to heat, as evidenced by the experiments of Fischer and Horbach (31) and Hasenclever and Mitchell (40) who used heat killed cells of *Candida* and by own experiments in which heat-treated extract of *Candid* was ffective (Figure 4)

(i) ( ) *Eff ct of Dead Candid or its Product on Resistance to other Microorganisms*

Roncidlo et al. (121 122) have prepared material from *Candid albicans* by treating yeast cells with trichloracetic

of milk has been reported in relation to bacterial infections (8)

The experiments in this group demonstrate enhancement of *Candida* infections by the injection of heat killed cells of a gram negative organism, *Escherichia coli* or its endotoxin given shortly after the infection. Own experiments with *E coli* endotoxin confirm these findings (Figure 7) and furnish supplemental data concerning the dependence of the effect on the time of administration of the endotoxin (Figures 2 5 6 and 8) Partial protection of mice pretreated 1 or 6 days before challenge with endotoxin of *Salmonella enteritidis* or *S typhosa* was also demonstrated and is in agreement with own findings (Figures 2 5 and 6) No protection was noted however when the interval was 3 10 or 14 days. Repeated injections of the endotoxins given 7 6 and 1 day before challenge offered a somewhat better protection.

Nonviable *Mycobacterium butyricum* given intraperitoneally 6 days before challenge with *Candida* did not prolong the survival time while the complete Freund's adjuvant containing this organism in oil offered some protection

Other experiments in this group demonstrated enhancement of *Candida* infections by injections of dead cells of a nonpathogenic fungus *Saccharomyces cerevisiae* given shortly after an infection with *Candida* and a partial protection against a *Candida* infection by injections of an extract from this yeast malucidin given 24 hours or 48 hours before challenge. Disintegrated spherules of a pathogenic fungus, *Coccidioides immitis* given several days before challenge with *Candida* protected mice somewhat against this challenge. The dependence of the effect on the time of administration of the mentioned fungal materials was in agreement with own experiments in which *E coli* endotoxin and *Candida* extract were used (Figures 2, 3 4 7 8 and 9) Sterile milk given 24 hours before challenge with *Candida* also caused a prolongation of life in mice

(1) (b) *Effect of Dead Candida or its Products on Nonspecific Resistance to Candida Infections*

Scherr (129) injected mice repeatedly with 10^9 formalin treated cells of *Candida albicans* intraperitoneally applying the same schedule as in a previously described experiment with formalin-treated cells of *Saccharomyces cerevisiae* (page 18) The injections were started 24 hours after an intraperitoneal injection of 5×10^7 cells of *C albicans* The resulting degree of dissemination of the *Candida* infection was much more pronounced in mice injected with formalin-treated *C albicans* than in untreated controls which received the infecting dose of *Candida* only

Fischer and Horbach (31) pretreated 30 mice with 0.2 cc of a 4 % suspension of heat killed cells intraperitoneally 24 hours before they challenged them by the same route with a suspension of living *Candida* containing the same number of cells as the first injection Ten of them died in 3 weeks while there were 15 deaths among 30 controls which received the challenge dose only and deaths among 15 mice receiving pretreatment only and no challenge

of this material. This impression seems supported by the finding of Yamabayashi that a *Candida* extract enhanced the bacterial challenge only when injected within about 12 hours preceding it, signifying possibly that the resistance influencing substance was more promptly vailable from the extract than from intact dead cells. Unlike Rusocillo et al. (120, 1 1 122) Yamabayashi (15) found this mechanism to be operative only in gram-negative but not in gram-positive bacterial organisms. In the experience of Yamabayashi the growth of *Staphylococcus* or *Str ptococcus* was not enhanced.

Mankiewicz and Liivak (78) have inoculated by subcutaneous route 20 guinea pigs simultaneously with *Mycobacterium t berculosis* and 1 ml. of a 5 % solution of a heat-stable polysaccharide fraction of *Candid albicans*. This was prepared by chemical extraction from supernatant after mechanical disintegration of the yeast cells of the fungus. Twenty controls were injected with *M t berculosis* alone.

Six of 20 guinea pigs injected with *M tuberculosis* and the *Candida* polysaccharide died within 6 weeks while 2 of 20 animals injected with *M tuberculosis* alone died f an unrelated bacterial infection. The rate of tissue invasion with *M t berculosis* in animals injected with the *Candid* polysaccharide and *M t ber ulosis* was higher than in those inoculated with the tubercle bacilli alone.

Mankiewicz and Liivak (78) reported that the chemical nature of the polysaccharide from *C albicans* was similar to bacterial dextrans which were shown to stimulate the growth of enterobacteria in vivo.

Mankiewicz, Stackiewicz and Liivak (79) also found that the polysaccharide fraction of *C albicans* promoted the growth of *M t berculosis* in vitro.

It is possible that the shortened survival time f guinea pigs injected with *M tuberculosis* and the polysaccharide f *C albicans* may have been due to the presence of an endotoxin-like substance of *Candida* which, as in previously described experiments with other bacterial organisms, may ha e decreased the resistance of the animals to *M tuberculosis*. It is also possible that the growth of the tubercle bacillus may have been promoted by another mechanism as well, which was independent of lowering of nonspecific resistance by an endotoxin-like substance in vivo and may have been based on the in vitro growth-promoting effect of the polysaccharide fraction of *C albicans* described by Mankiewicz, Stackiewicz and Liivak (79)

The experiments in this group demonstrate that an extract of *Candida albicans* injected simultaneously with *Salmonella typhosa* or *Staphylococcus aureus* or an extract of *Candid Krusei* injected simultaneously with *Staphylococcus aureus* in the experiments of Rusocillo et al. (120, 121 122) enhances the bacterial infection. Heat-killed cells of *C albicans* in the experiments of Yamabayashi (152) injected less than 48 hours before challenge with *Proteus* or *Pseudomonas* or an extract of the fungus prepared by trypsin digestion and

acid, neutralizing them with sodium hydroxide and precipitating with acetone. Extraction of insoluble polysaccharides was done with Na_2HPO_4. After filtering and discarding of the solution the remaining yeast was suspended in water and alkalized with sodium hydroxide. Digestion of the remainder was done with trypsin and the sediment was boiled in water. Boiling in water was repeated after suspension of the sediment in alcohol. The dry residue was ground in a mortar. This material which the authors called zymosan presumably originated in cell walls of *Candida*.

Mice were injected with mixtures of 5 mg of this material and varying numbers of 1 million to 2,000 million cells of *Salmonella typhosa* intraperitoneally. In a control group only 2 000 million bacteria were lethal to mice. In the groups injected with the mixtures of *Salmonella* and the extract from *Candida* even mice receiving the smallest doses of bacteria died.

In another series of experiments mice were injected with a mixture of *Staphylococcus aureus* and 5 mg of the described *Candida* extract. All these mice died within the first 24 hours while in a control group of mice receiving *Candida* extract only all animals survived.

Rzucidlo Stachów Nowakowska and Kubica (120) injected 5 mg of a similarly prepared extract from *Candida Krusei* together with a suspension of *Staphylococcus aureus* intraperitoneally into mice. All 10 mice injected with this mixture died within 18 hours while 10 controls receiving *Staphylococcus* alone survived for more than 48 hours. Mice receiving the cell extract of *C Krusei* alone remained alive during the 10 day observation period.

Yamabayashi (157) injected a suspension of 50×10^7 heat killed cells of *Candida albicans* which was followed 2 to 48 hours later by the administration of 1/10 of the minimal lethal dose of either *Proteus* or *Pseudomonas*. The death rates were highest when bacterial organisms were injected 2 hours after *Candida* and almost no effect on the death of mice was seen when bacterial organisms were injected 48 hours after heat killed *Candida*.

Yamabayashi also prepared a zymosan-like substance from *C albicans* by trypsin digestion. It was a light-gray powder insoluble in water mainly composed of carbohydrate. Mice were injected with 2 mg of this substance intraperitoneally and the injection was followed 1/2 to 48 hours later by an intraperitoneal administration of *Proteus vulgaris Pseudomonas aeruginosa* or *Escherichia coli*. The death rates were very high when mice were infected up to 12 hours after the injection of the *Candida* extract. This effect was less pronounced with *E coli* than it was with the first two organisms. No effects were seen after injections of *Staphylococcus aureus* or *Streptococcus haemolyticus*.

The finding that dead *Candida* cells were effective in enhancing the bacterial infection when given up to about 48 hours before bacterial challenge may be due to the possibility that the endotoxin like substance may be released rather slowly from dead cells, and that peak blood levels of endotoxin may be reached many hours after the injection

of this material. This impression seems supported by the finding of Yamabayashi that a *Candida* extract enhanced the bacterial challenge only when injected within about 12 hours preceding it, signifying possibly that the resistance influencing substance was more promptly available from the extract than from intact dead cells. Unlike Rmcadlo et al. (120 121 122) Yamabayashi (152) found this mechanism to be operative only in gram-negative but not in gram-positive bacterial organisms. In the experience of Yamabayashi the growth of *St phy ococcus* or *Streptococcus* was not enhanced.

Mankiewicz and Liivak (78) have inoculated by subcutaneous route 20 guinea pigs simultaneously with *Mycobacterium tuberculosis* and 1 ml. of a 5 % solution of heat-stable polysaccharide fraction of *Candid albicans*. This was prepared by chemical extraction from a supernatant after mechanical disintegration of the yeast cells of the fungus Twenty controls were injected with *M t ber ulosis* alone

Six of 20 guinea pigs injected with *M t be cul si* and the *Candid* polysaccharide died within 6 weeks while 2 of 20 animals injected with *M t ber ulosis* alone died of an unrelated bacterial infection. The rate of tissue invasion with *M t berculosis* in animals injected w th the *Candida* polysaccharide and *M t be culosis* was higher than in those inoculated with the tubercle bacilli alone

Mankiewicz and Litvak (78 reported that the chemical nature of the polysaccharide from *C albicans* was similar to bacterial dextrans which were shown to stimulate the growth of enterobacteria in vivo.

Mankiewicz, Stackiewicz and Litvak (79) also found that the polysaccharide fraction of *C albicans* promoted the growth of *M tuberculosis* in vitro.

It is possible that the shortened survival time of guinea pigs injected with *M tube culosis* and the polysaccharide of *C albicans* may have been due to the presence of an endotoxin-like substance of *Candida* which, as in previously described experiments with other bacterial organisms, may have decreased the resistance of the animals to *M tuber ulosis*. It is also possible that the growth of the tubercle bacillus may have been promoted by another mechanism as well, which was independent of lowering of nonspecific resistance by an endotoxin-like substance in vivo and may have been based on the in vitro growth-promoting effect of the polysaccharide fraction of *C albicans* described by Mankiewicz, Stackiewicz and Litvak (79)

The experiments in this group demonstrate that an extract of *Candida albicans* injected simultaneously with *Salmonella typhosa* or *Staphylococcus au eus* or an extract of *Cand d Krusei* injected simultaneously with *Staphylococcus au eus* in the experiments of Rmcadlo et al. (120, 121 122) enhances the bacterial infection. Heat killed cells of *C albicans* in the experiments of Yamabayashi (152) injected less than 48 hours before challenge with *P oteus* or *Pseudomonas*, or an extract of the fungus prepared by trypsin digestion and

acid, neutralizing them with sodium hydroxide and precipitating with acetone. Extraction of insoluble polysaccharides was done with Na HPO$_4$. After filtering and discarding of the solution the remaining yeast was suspended in water and alkalized with sodium hydroxide. Digestion of the remainder was done with trypsin and the sediment was boiled in water Boiling in water was repeated after suspension of the sediment in alcohol. The dry residue was ground in a mortar This material which the authors called zymosan presumably originated in cell walls of *Candida*.

Mice were injected with mixtures of 5 mg of this material and varying numbers of 1 million to 2 000 million cells of *Salmonella typhosa* intraperitoneally In a control group only 2 000 million bacteria were lethal to mice. In the groups injected with the mixtures of *Salmonella* and the extract from *Candida* even mice receiving the smallest doses of bacteria died.

In another series of experiments mice were injected with a mixture of *Staphylococcus aureus* and 5 mg of the described *Candida* extract. All these mice died within the first 24 hours while in a control group of mice receiving *Candida* extract only all animals survived

Rzucidlo Stachów Nowakowska and Kubica (120) injected 5 mg of a similarly prepared extract from *Candida Krusei* together with a suspension of *Staphylococcus aureus* intraperitoneally into mice. All 10 mice injected with this mixture died within 18 hours while 10 controls receiving *Staphylococcus* alone survived for more than 48 hours.

Mice receiving the cell extract of *C Krusei* alone remained alive during the 10 day observation period

Yamabayashi (152) injected a suspension of 50 $\times$ 10^7 heat killed cells of *Candida albicans* which was followed 2 to 48 hours later by the administration of 1/10 of the minimal lethal dose of either *Proteus* or *Pseudomonas* The death rates were highest when bacterial organisms were injected 2 hours after *Candida* and almost no effect on the death of mice was seen when bacterial organisms were injected 48 hours after heat killed *Candida*

Yamabayashi also prepared a zymosan-like substance from *C albicans* by trypsin digestion. It was a light-gray powder insoluble in water mainly composed of carbohydrate. Mice were injected with 2 mg of this substance intraperitoneally and the injection was followed 1/2 to 48 hours later by an intraperitoneal administration of *Proteus vulgaris Pseudomonas aeruginosa* or *Escherichia coli* The death rates were very high when mice were infected up to 12 hours after the injection of the *Candida* extract This effect was less pronounced with *E coli* than it was with the first two organisms. No effects were seen after injections of *Staphylococcus aureus* or *Streptococcus haemolyticus*

The finding that dead *Candida* cells were effective in enhancing the bacterial infection when given up to about 48 hours before bacterial challenge may be due to the possibility that the endotoxin like substance may be released rather slowly from dead cells, and that peak blood levels of endotoxin may be reached many hours a[illegible]

substances which may have mutually stimulated the growth of the injected microorganisms by the mechanism of nonspecific immunity. These findings are in agreement with the results of Yamabayashi (152) who confirmed and amplified them by including some additional organisms in his tests. Yamabayashi injected simultaneously groups of 20—30 mice by intraperitoneal route with varying amounts of *Candida albicans* and 1/10 of the minimal lethal dose of either *Proteus vulgaris*, *Pseudomonas aeruginosa* or *Escherichia coli*. All death rates were computed 7 days after the injections. The author found that the death rates of mice with simultaneous infections were considerably higher than those in which *Candida* alone was injected. This effect was more pronounced with *Proteus* and *Pseudomonas* than with *E. coli*. When gram-positive organisms, viz. *Staphylococcus aureus*, *Streptococcus haemolyticus* or *Bacillus subtilis* were injected simultaneously with *Candida* no increase of death rates was found.

In another series of experiments *Proteus* or *Pseudomonas* were injected at intervals between 2 and 48 hours after an injection of *Candida*. The death rates were highest when bacterial organisms were injected 2 hours after *Candida* and almost no effect on the death of mice was seen when bacterial organisms were injected 48 hours after *Candida*.

In vitro experiments have shown that there was no difference in colony counts between the mixed cultures and cultures of individual organisms.

The finding of Yamabayashi (152) that the mixed infection of *Candida* and *Escherichia coli* caused fewer deaths than that of *Candida* and *Proteus* or *Pseudomonas* is further elucidated by the experiments of Gale and Sandoval (37). These authors injected into mice sublethal amounts of living *Escherichia coli* before or after an injection of a lethal concentration of *Candida*. The injections of *Candida* were always given intravenously, those of *E. coli* either intraperitoneally or intravenously.

Intravenous inoculation of 1.8×10^{8} of *Candida* alone killed all mice within 24 hours. When 3.7×10^{7} cells of *E. coli* were injected intravenously 30 minutes before the lethal *Candida* injection, 3 of 7 mice survived for 6 days, at which time they were sacrificed. When *E. coli* was given intraperitoneally under otherwise identical conditions 2 of 7 mice survived for 6 days. Intravenous inoculation of *E. coli* after the *Candida* injection was ineffectual in delaying the time of death, while some protective activity was produced by the intraperitoneal inoculation of *E. coli* 30 or 150 minutes after *Candida*. This protective activity was produced by *E. coli* only within a narrow range of concentration (10^{7} and 10^{8}). For all concentrations of *E. coli* the survival time after administration of dead bacilli was shorter than after administration of the living organisms (page 17).

Gale and Sandoval (37) found also that *E. coli* was very effective in inhibiting the *in vitro* growth of *Candida albicans*. The greather the concentration of *E. coli* the more effective was the inhibition of *Candida*.

It appears probable that an antibiotic substance produced by *E. coli* may be

injected less than 12 hours before challenge with *Proteus Pseudomonas* or *E. coli* enhances the bacterial infection with the former 2 organisms and to a lesser extent with *E coli*. Yamabayashi found no effect on the infection with *Staphylococcus* or *Streptococcus* A simultaneous injection of a soluble polysaccharide of *C albicans* enhanced the tuberculous infection in the experiments of Mankiewicz and Liivak (78)

It is possible that at least a part of the enhancing effect in these experiments, with the limitations mentioned earlier was due to the endotoxin-like substance of *C albicans* or a similar substance of *C Krusei* A similar effect was illustrated by own experiments in which the survival time of mice injected with the soluble extract of *C albicans* 2 hours before challenge with *Salmonella enteritidis* was shorter than in the controls (Figure 12)

As long however as the chemical nature of the own endotoxin-like substance of *Candida* and of the various above mentioned extracts is unknown it is impossible to determine whether the chemically extracted preparations of Rzucidlo et al. (120 121 122) and Yamabayashi (152) actually contained the same resistance influencing substance which is present in the own soluble extract from *Candida*. The preparation of Mankiewicz and Liivak (78) was, similarly as the own soluble extract, prepared from the supernatant remaining after disintegration of yeast cells, so that most of the cell wall material was apparently excluded from both preparations.

(ii) (a) *Effect of Double Infections with Candida and Another Organism on Nonspecific Resistance*

When evaluating the following group of experiments it has to be realized that since the two organisms are living both may be capable of producing constantly an endotoxin-like substance. They may however also produce antibiotic substances which may influence the development of the mixed infection.

Donomae and Kawamori (18) infected mice simultaneously with 50 / 10^6 cells of *Candida albicans* and 1/10 of the minimal lethal dose of *Proteus vulgaris* About 50 % of controls injected with *Candida* only were dead at the end of 7 days while all mice with the mixed infection succumbed in 3 days. Another group of mice was infected simultaneously with 100 / 10^6 cells of *C albicans* and 1/20 of the minimal lethal dose of *Proteus vulgaris* About 20 % of controls injected with *Candida* only were dead after 7 days while all mice with the mixed infection died in 3 days Similar results were obtained in a mixed infection with *C albicans* and *Pseudomonas aeruginosa*. Death rates of mice injected simultaneously with *C albicans* and 1/10 of the minimal lethal dose of either *Staphylococcus Streptococcus hæmolyticus Escherichia coli* or *Bacill[illegible] [illegible]btilis* did not differ from those [illegible] [illegible]ntr[illegible]l which received *C albicans* only

The enhancement in a simult[illegible]ne[illegible] infection with *Candida* and *P[illegible]* [illegible] *Pseudomonas* was apparently [illegible] [illegible] liberation, by both *Candida* [illegible]nd the bacterial organi[illegible]m [illegible] of endo[illegible]in-lik[illegible]

substances which may have mutually stimulated the growth of the injected microorganisms by the mechanism of nonspecific immunity. These findings are in agreement with the results of Yamabayashi (152) who confirmed and amplified them by including some additional organisms in his tests. Yamabayashi injected simultaneously groups of 20—30 mice by intraperitoneal route with varying amounts of *Candida albicans* and 1/10 of the minimal lethal dose of either *Proteus vulgaris, Pseudomonas aeruginosa* or *Escherichia coli*. All death rates were computed 7 days after the injections. The author found that the death rates of mice with simultaneous infections were considerably higher than those in which *Candida* alone was injected. This effect was more pronounced with *Proteus* and *Pseudomonas* than with *E. coli*. When gram-positive organisms, viz. *Staphylococcus aureus, Streptococcus haemolyticus* or *Bacillus subtilis* were injected simultaneously with *Candida* no increase of death rates was found.

I another series of experiments *Proteus* or *Pseudomonas* were injected at intervals between 2 and 48 hours after an injection of *Candida*. The death rates were highest when bacterial organisms were injected 2 hours after *Candida* and almost no effect on the death of mice was seen when bacterial organisms were injected 48 hours after *Candida*.

In vitro experiments have shown that there was no difference in colony counts between the mixed cultures and cultures of individual organisms.

The finding of Yamabayashi (152) that the mixed infection of *Candida* and *Escherichia coli* caused fewer deaths than that of *Candida* and *Proteus* or *Pseudomonas* is further elucidated by the experiments of Gale and Sandoval (57). These authors injected into mice sublethal amounts of living *Escherichia coli* before or after an injection of a lethal concentration of *Candida*. The injections of *Candida* were always given intravenously; those of *E. coli* either intraperitoneally or intravenously.

Intravenous inoculation of 1.8×10^{8} of *Candida* alone killed all mice within 24 hours. When 3.7×10^{5} cells of *E. coli* were injected intravenously 30 minutes before the lethal *Candida* injection, 3 of 7 mice survived for 6 days, at which time they were sacrificed. When *E. coli* was given intraperitoneally under otherwise identical conditions 2 of 7 mice survived for 6 days. Intravenous inoculation of *E. coli* after the *Candida* injection was ineffectual in delaying the time of death while some protective activity was produced by the intraperitoneal inoculation of *E. coli* 30 or 150 minutes after *Candida*. This protective activity was produced by *E. coli* only within a narrow range of concentration (10 and 10^{8}). For all concentrations of *E. coli* the survival time after administration of dead bacilli was shorter than after administration of the living organisms (page 17).

Gale and Sandoval (57) found also that *E. coli* was very effective in inhibiting the in vitro growth of *Candida albicans*. The greater the concentration of *E. coli* the more effective was the inhibition of *Candida*.

It appears probable that an antibiotic substance produced by *E. coli* may be

injected less than 12 hours before challenge with *Proteus Pseudomonas* or *E. coli* enhances the bacterial infection with the former 2 organisms, and to a lesser extent with *E. coli*. Yamabayashi found no effect on the infection with *Staphylococcus* or *Streptococcus*. A simultaneous injection of a soluble polysaccharide of *C. albicans* enhanced the tuberculous infection in the experiments of Mankiewicz and Liivak (78).

It is possible that at least a part of the enhancing effect in these experiments, with the limitations mentioned earlier was due to the endotoxin like substance of *C. albicans* or a similar substance of *C. Krusei*. A similar effect was illustrated by own experiments in which the survival time of mice injected with the soluble extract of *C. albicans* 2 hours before challenge with *Salmonella enteritidis* was shorter than in the controls (Figure 12).

As long however as the chemical nature of the own endotoxin like substance of *Candida* and of the various above mentioned extracts is unknown it is impossible to determine whether the chemically extracted preparations of Raucidlo et al. (120 121 122) and Yamabayashi (152) actually contained the same resistance-influencing substance which is present in the own soluble extract from *Candida*. The preparation of Mankiewicz and Liivak (78) was, similarly as the own soluble extract prepared from the supernatant remaining after disintegration of yeast cells, so that most of the cell wall material was apparently excluded from both preparations.

(ii) (a) *Effect of Double Infections with Candida and Another Organism on Nonspecific Resistance*

When evaluating the following group of experiments it has to be realized that since the two organisms are living both may be capable of producing constantly an endotoxin like substance. They may however also produce antibiotic substances which may influence the development of the mixed infection.

Donomae and Kawamori (18) infected mice simultaneously with 50×10^6 cells of *Candida albicans* and 1/10 of the minimal lethal dose of *Proteus vulgaris*. About 50 % of controls injected with *Candida* only were dead at the end of 7 days while all mice with the mixed infection succumbed in 3 days. Another group of mice was infected simultaneously with 100×10^6 cells of *C. albicans* and 1/20 of the minimal lethal dose of *Proteus vulgaris*. About 20 % of controls injected with *Candida* only were dead after 7 days while all mice with the mixed infection died in 3 days. Similar results were obtained in a mixed infection with *C. albicans* and *Pseudomonas aeruginosa*. Death rates of mice injected simultaneously with *C. albicans* and 1/10 of the minimal lethal dose of either *Staphylococcus Streptococcus haemolyticus Escherichia coli* or *Bacillus subtilis* did not differ from those of controls which received *C. albicans* only.

The enhancement in a simultaneous infection with *Candida* and *Proteus* or *Pseudomonas* was apparently due to liberation, by both *Candida* and the bacterial organisms, of endotoxin-like

peritoneally with 5×10^{6} cells of *Candida albicans*. Twenty four hours later intraperitoneal injections of 10^{5} cells of *Saccharomyces cerevisiae* were started and repeated every other day for a total of 10 injections. When the mice were sacrificed 20 days after the injection of *Candida* it was found that the degree of dissemination of moniliasis was much more pronounced in animals treated with *Saccharomyces* than in untreated controls which received only *Candida*.

In another experiment Scherr studied the effect of one single injection of *Sac ha omyces* containing the total number of living cells that were used in all 10 injections in the preceding experiment combined. Such an injection was given either 24 hours prior to the *Ca did* infection together with it or 4 48 or 72 hours later While there was no essential difference between controls and treated mice when *Saccharomyces* was injected 24 hours before the infection, a markedly increased dissemination was noted in all other groups of mice, particularly in those that re ceived yeast together with or 24 hours after the infection.

Hasenclever and Mitchell (40) injected mice intraperitoneally with either 10^{6} cells of *Histoplasma capsulat m* 10^{7} cells of *Cryptococcus eoformans* 4×10^{4} spherule units of *Coccidioides im mitis* or 10^{5} conidiospores of *Aspergillus f m gatus* six days before they challenged them intra enously with 10^{7} cells of *Candida albicans* The life of pre treated mice was somewhat prolonged as compared to mice which received *Ca dida* hallenge only

The experiments of Scherr (129) and of Hasenclever and Mitchell (40) demonstrate that modification of the infection may take place when the two viable organisms in the experiment are fungi, pathogenic or not. The modifying influence may have been exerted by resistance-influencing substances of *Saccharomyc s cerevisiae Hist plasma capsulatum Cryptococcus eof rmans Coccidioides immitis* and *Aspergillus fumigatus*.

The experiments in this group demonstrate modifications in the course of infections in animals injected with two living bacterial or fungal microorganisms, one of which is *Candida*. The results show that in simultaneous infections with *Candida albicans* and *Proteus* or *Pseudomo as* the mortalities of mice are much higher than in infections with single organisms. When these bac teria were injected 2—24 hours after *Ca dida* the death rates were highest in mice injected with bacterial organisms 2 hours after *Candida*. The enhancing effect in these experiments was appar ently due to the presence in both *Can dida* and the bacterial organisms of endotoxin-like substances capable of reducing resistance to the injected organisms, which was demonstrated in own ex periments (Figures 7 8, 9 and 12) Experiments of others with dead organisms or their products described in Section (1)(a) and ( )(c) are in general agreement with these results. No increase in death rates was found when gram-positive organisms were injected simultaneously with *Candida*. When *Es herichia oli* was used the increase in mor tality rates was less pronounced. This may hav been due to the production by

responsible for this protective activity against *Candida* especially since this influence was absent when heat killed cells of *E coli* were used.

The finding of Gale and Sandoval (37) that *E coli* given intraperitoneally 30 or 150 minutes after *Candida* protected some mice from death, but only when the dosage was 10^{8} or 10^{7} cells, appears to be in agreement with this idea. It is possible that with a lower dosage the antibiotic effect did not take place because of a too small number of *E coli* capable of producing the hypothetic antibiotic substance, and with a higher dosage this antibiotic effect may have been nullified by the influence of the infection-enhancing effect of the endotoxin of *E coli* which may have been ineffective at a lower dose.

In support of this concept is also the finding of the same investigators that *E coli* inhibits *Candida* in vitro the experimental results of Banič (2) who found a substance in *E coli* which exerted a fungistatic effect on *C albicans* and the report of Freyschuss, Pehrson and Steenberg (35) who isolated coliformin an antibiotic from *E coli* which is active against *C albicans* and several other fungi. Similar work was also done by Wiedling (150) Paine (101) Rosebury Gale and Taylor (113) and Fischer (30) who observed inhibition of *C albicans* by *E. coli*

Mankiewicz and Livak (78) inoculated 20 guinea pigs simultaneously with *Mycobacterium tuberculosis* and with 1 mg (wet weight) of *Candida albicans* by subcutaneous route. Twenty animals were inoculated with tubercle bacilli alone and kept as controls. An additional control group of 20 guinea pigs was given 1 mg of *C albicans* alone. Another group was inoculated with *M tuberculosis* and a polysaccharide fraction of *C albicans* (page 23)

Eleven of 20 guinea pigs inoculated with *M tuberculosis* and *C albicans* died within 6 weeks. During the same period of time 1 of 20 guinea pigs injected with *Candida* alone and 2 of 20 animals inoculated with *M tuberculosis* alone died of unrelated bacterial infections.

On autopsies of surviving animals performed in the twelfth week after inoculation it was found that cultures of the liver spleen and lungs of guinea pigs inoculated with the two microorganisms were much more frequently positive for *M tuberculosis* than the organs of guinea pigs inoculated with *M tuberculosis* alone *Candida* was isolated from regional lymph nodes but not from internal organs of guinea pigs with the simultaneous infection in the first 6 weeks. After this time *Candida* was not cultured from any tissue.

The shortened survival time resulting from a simultaneous infection with *C albicans* and *M tuberculosis* may have been due to the presence of a resistance influencing substance of *C albicans* and possibly a similar substance in *M tuberculosis* (127) It is also possible that the growth of *M tuberculosis* may have been promoted by another mechanism (page 23) viz. an *in* vitro growth-promoting effect of the polysaccharide fraction of *C albicans* as described by Mankiewicz, Stackiewicz and Livak (79)

Scherr (129) injected mice intra

peritoneally with 5×10^6 cells of *Candida albicans*. Twenty-four hours later intraperitoneal injections of 10^6 cells of *Saccharomyces cerevisiae* were started and repeated every other day for a total of 10 injections. When the mice were sacrificed 20 day after the injection of *Candida* it was found that the degree of dissemination of moniliasis was much more pronounced in animals treated with *Saccharomyces* than in untreated controls which received only *Candida*.

In another experiment Scherr studied the effect of one single injection of *Saccharomyces* containing the total number of living cells that were used in all 10 injections in the preceding experiment combined. Such an injection was given either 24 hours prior to the *Candida* infection, together with it or 4 48 or 72 hours later. While there was no essential difference between controls and treated mice when *Saccharomyces* was injected 24 hours before the infection, a markedly increased dissemination was noted in all other groups of mice particularly in those that received yeast together with or 24 hours after the infection.

Hasenclever and Mitchell (40) injected mice intraperitoneally with either 10^6 cells of *Histoplasma capsulatum* 10^7 cells of *Cryptococcus neoformans* 4×10 spherules of *Coccidioides immitis* or 10 conidiospores of *Aspergillus fumigatus* six days before they challenged them intravenously with 10^5 cells of *Candida albicans*. The ht of pre treated mic was somewhat prolonged as compared to mice which received *Candida* challenge only.

The experiments of Scherr (129) and of Hasenclever and Mitchell (40) demonstrate that modification of the infection may take place when the two viable organisms in the experiment are fungi, pathogenic or not. The modifying influence may have been exerted by resistance-influencing substances of *Saccharomyces cerevisiae*, *Histoplasma capsulatum*, *Cryptococcus neoformans*, *Coccidioides immitis* and *Aspergillus fumigatus*.

The experiments in this group demonstrate modifications in the course of infections in animals injected with two living bacterial or fungal microorganisms, one of which is *Candida*. The results show that in simultaneous infections with *Candida albicans* and *Proteus* or *Pseudomonas* the mortalities of mice are much higher than in infections with single organisms. When these bacteria were injected 2—24 hours after *Candida* the death rates were highest in mice injected with bacterial organisms 2 hours after *Candida*. The enhancing effect in these experiments was apparently due to the presence in both *Candida* and the bacterial organisms of endotoxin-like substances capable of reducing resistance to the injected organisms, which was demonstrated in own experiments (Figures 7 8, 9 and 12). Experiments of others with dead organisms or their products described in Section ( )(a) and (I)( ) are in general agreement with these results. No increase in death rates was found when gram-positive organisms were injected simultaneously with *Candida*. When *Escherichia coli* was used the increase in mortality rates was less pronounced. This may hav been due to the production by

responsible for this protective activity against *Candida* especially since this influence was absent when heat killed cells of *E. coli* were used.

The finding of Gale and Sandoval (37) that *E. coli* given intraperitoneally 30 or 150 minutes after *Candida* protected some mice from death, but only when the dosage was 10^6 or 10^7 cells, appears to be in agreement with this idea. It is possible that with a lower dosage the antibiotic effect did not take place because of a too small number of *E. coli* capable of producing the hypothetic antibiotic substance, and with a higher dosage this antibiotic effect may have been nullified by the influence of the infection-enhancing effect of the endotoxin of *E. coli* which may have been ineffective at a lower dose.

In support of this concept is also the finding of the same investigators that *E. coli* inhibits *Candida* in vitro, the experimental results of Banič (2) who found a substance in *E. coli* which exerted a fungistatic effect on *C. albicans* and the report of Freyschuss, Pehrson and Steenberg (35) who isolated coliformin an antibiotic from *E. coli* which is active against *C. albicans* and several other fungi. Similar work was also done by Wiedling (150) Paine (101) Rosebury, Gale and Taylor (113) and Fischer (30) who observed inhibition of *C. albicans* by *E. coli*.

Mankiewicz and Liivak (78) inoculated 20 guinea pigs simultaneously with *Mycobacterium tuberculosis* and with 1 mg (wet weight) of *Candida albicans* by subcutaneous route. Twenty animals were inoculated with tubercle bacilli alone and kept as controls. An additional control group of 20 guinea pigs was given 1 mg of *C. albicans* alone. Another group was inoculated with *M. tuberculosis* and a polysaccharide fraction of *C. albicans* (page 23).

Eleven of 20 guinea pigs inoculated with *M. tuberculosis* and *C. albicans* died within 6 weeks. During the same period of time 1 of 20 guinea pigs injected with *Candida* alone and 2 of 20 animals inoculated with *M. tuberculosis* alone died of unrelated bacterial infections.

On autopsies of surviving animals performed in the twelfth week after inoculation it was found that cultures of the liver, spleen and lungs of guinea pigs inoculated with the two microorganisms were much more frequently positive for *M. tuberculosis* than the organs of guinea pigs inoculated with *M. tuberculosis* alone. *Candida* was isolated from regional lymph nodes but not from internal organs of guinea pigs with the simultaneous infection in the first 6 weeks. After this time *Candida* was not cultured from any tissue.

The shortened survival time resulting from a simultaneous infection with *C. albicans* and *M. tuberculosis* may have been due to the presence of a resistance-influencing substance of *C. albicans* and possibly a similar substance in *M. tuberculosis* (127). It is also possible that the growth of *M. tuberculosis* may have been promoted by another mechanism (page 23), viz. an in vitro growth-promoting effect of the polysaccharide fraction of *C. albicans* as described by Mankiewicz, Stackiewicz and Liivak (79).

Scherr (129) injected mice intra

peritoneally with 5×10^6 cells of *Candida albicans*. Twenty-four hours later intraperitoneal injections of 10^6 cells of *Saccharomyces cerevisiae* were started and repeated every other day for a total of 10 injections. When the mice were sacrificed 20 days after the injection of *Candida* it was found that the degree of dissemination of moniliasis was much more pronounced in animals treated with *Saccharomyces* than in untreated controls which received only *Candida*.

In another experiment Scherr studied the effect of one single injection of *Saccharomyces* containing the total number of living cells that were used in all 10 injections in the preceding experiment combined. Such an injection was given either 24 hours prior to the *Candida* infection, together with it or 4, 48 or 72 hours later. While there was no essential difference between controls and treated mice when *Saccharomyces* was injected 24 hours before the infection, a markedly increased dissemination was noted in all other groups of mice, particularly in those that received yeast together with or 24 hours after the infection.

Hasenclever and Mitchell (40) injected mice intraperitoneally with either 10^6 cells of *Histoplasma capsulatum*, 10^7 cells of *Cryptococcus neoformans*, 4×10 spherule units of *Coccidioides immitis* or 10^7 conidiospores of *Aspergillus fumigatus* six days before they challenged them intravenously with 10^7 cells of *Candida albicans*. The life of pretreated mice was somewhat prolonged as compared to mice which received *Candida* challenge only.

The experiments of Scherr (129) and of Hasenclever and Mitchell (40) demonstrate that modification of the infection may take place when the two viable organisms in the experiment are fungi, pathogenic or not. The modifying influence may have been exerted by resistance-influencing substances of *Saccharomyces cerevisiae*, *Histoplasma capsulatum*, *Cryptococcus neoformans*, *Coccidioides immitis* and *Aspergillus fumigatus*.

The experiments in this group demonstrate modifications in the course of infections in animals injected with two living bacterial or fungal microorganisms, one of which is *Candida*. The results show that in simultaneous infections with *Candida albicans* and *Proteus* or *Pseudomonas* the mortalities of mice are much higher than in infections with single organisms. When these bacteria were injected 2—24 hours after *Candida* the death rates were highest in mice injected with bacterial organisms 2 hours after *Candida*. The enhancing effect in these experiments was apparently due to the presence in both *Candida* and the bacterial organisms of endotoxin-like substances capable of reducing resistance to the injected organisms, which was demonstrated in own experiments (Figures 7, 8, 9 and 12). Experiments of others with dead organisms or their products described in Section (1)(a) and (1)(c) are in general agreement with these results. No increase in death rates was found when gram-positive organisms were injected simultaneously with *Candida*. When *Escherichia coli* was used the increase in mortality rates was less pronounced. This may have been due to the production by

responsible for this protective activity against *Candida* especially since this influence was absent when heat killed cells of *E coli* were used.

The finding of Gale and Sandoval (37) that *E coli* given intraperitoneally 30 or 150 minutes after *Candida* protected some mice from death, but only when the dosage was 10^6 or 10^7 cells, appears to be in agreement with this idea. It is possible that with a lower dosage the antibiotic effect did not take place because of a too small number of *E coli* capable of producing the hypothetic antibiotic substance, and with a higher dosage this antibiotic effect may have been nullified by the influence of the infection-enhancing effect of the endotoxin of *E. coli* which may have been ineffective at a lower dose.

In support of this concept is also the finding of the same investigators that *E coli* inhibits *Candida* in vitro, the experimental results of Banič (2) who found a substance in *E coli* which exerted a fungistatic effect on *C albicans* and the report of Freyschuss, Pehrson and Steenberg (35) who isolated coliformin an antibiotic from *E coli* which is active against *C albicans* and several other fungi. Similar work was also done by Wiedling (150) Paine (101) Rosebury Gale and Taylor (113) and Fischer (30) who observed inhibition of *C albicans* by *E coli*.

Mankiewicz and Liivak (78) inoculated 20 guinea pigs simultaneously with *Mycobacterium tuberculosis* and with 1 mg (wet weight) of *Candida albicans* by subcutaneous route. Twenty animals were inoculated with tubercle bacilli alone and kept as controls. An additional control group of 20 guinea pigs was given 1 mg of *C albicans* alone. Another group was inoculated with *M tuberculosis* and a polysaccharide fraction of *C albicans* (page 23)

Eleven of 20 guinea pigs inoculated with *M tuberculosis* and *C albicans* died within 6 weeks. During the same period of time 1 of 20 guinea pigs injected with *Candida* alone and 2 of 20 animals inoculated with *M tuberculosis* alone died of unrelated bacterial infections.

On autopsies of surviving animals performed in the twelfth week after inoculation it was found that cultures of the liver spleen and lungs of guinea pigs inoculated with the two microorganisms were much more frequently positive for *M tuberculosis* than the organs of guinea pigs inoculated with *M tuberculosis* alone *Candida* was isolated from regional lymph nodes but not from internal organs of guinea pigs with the simultaneous infection in the first 6 weeks. After this time *Candida* was not cultured from any tissue.

The shortened survival time resulting from a simultaneous infection with *C albicans* and *M tuberculosis* may have been due to the presence of a resistance-influencing substance of *C albicans* and possibly a similar substance in *M tuberculosis* (127) It is also possible that the growth of *M tuberculosis* may have been promoted by another mechanism (page 23) viz. an in vitro growth promoting effect of the polysaccharide fraction of *C albicans* as described by Mankiewicz, Stackiewicz and Liivak (79)

Scherr (129) injected mice intra

days. By the time the second injection was administered in the experiment in which the interval between the two injections was 30 days the primary infectious process may have been subsiding and there was apparently not enough of the endotoxin-like substance produced in some animals to counteract the protective effect of the specific antibodies which may have developed in the meantime. The 2/3 that did die may have been rendered hypersensitive, their antibodies may not have been sufficient and the endotoxin-like substance may have stimulated the spread of the organisms in the second injection.

Fischer and Horbach (31) have pretreated 30 mice with 0.5 cc. of a 0 1 % suspension of living cells of *Candida albicans* (about 6,250,000 cells) intraperitoneally twenty-four hours before they challenged them by the same rout with 0 2 cc. of a 4 % suspension of living cells of the same fungus (about 100,000,000 cells) Nineteen mice died in 3 weeks. Among 30 controls which received the challenge dose only there were 15 deaths after 3 weeks. Of 15 controls receiving pretreatment only and no challenge 4 mice died.

In this experiment pretreatment with small number of cells was given 24 hours before a sizeable challenge. The number of mice dying after the two injections was slightly higher than that of control mice receiving challenge only A possible explanation of this relatively slight infection-enhancing ffect may be that the time between the injections was rather short to afford sufficient multiplication of the fungus which would liberate enough endotoxin-like substance which would enhance the growth of fungi contained in the challenge dose.

Hasenclever and Mitchell (40, 41) injected mice intraperitoneally with 10^7 cells of *Candida albicans* and challenged them 6 days later with the same number of cells intravenously They found that 80 % of the controls which received challenge only were dead 17 hours after the injection whereas only 10 % of the pretreated mice had succumbed during this period. All controls were dead at the end of 24 hours while all pretreated mice were dead at 72 hours. In experiments in which the interval between pretreatment and challenge was 1 3, 10 or 14 days there was no ppreciable change in mortality rates of mice. When the challenge dose was increased to 5×10^7 cells in mice pretreated with 10^7 viable cells intraperitoneally there was no significant difference between pretreated mice and controls. No effect on the survival times of mice was caused by pretreatment of mice with 2×10^7 of viable cells of *C albicans* given intraperitoneally 7 and again 4 days before challenge.

Pretreatment with living cells of members of the genus *Candida* other than albi ans (*C stellatoidea C tropicalis C Guilliermondi, C parapsilosis*) increased the survival time of mice challenged with *C albicans* similarly as pretreatment with *C albicans* but to a lesser extent.

The delayed time of death in mice pretreated intraperitoneally with living cells of *C albicans* 6 days prior to intra enous challenge with the same organism may be due to the fact that the infec

E coli of an antibiotic substance active against *C albicans* in vitro which was reported by several authors.

Experiments with a simultaneous infection with *C albicans* and *Mycobacterium tuberculosis* resulted in an enhancement of the tuberculous infection. This may have been based on the combination of the same mechanism as the enhancement in the simultaneous infections described above, and the promoting effect of the polysaccharide fractions of *C albicans* effective in vitro on *M tuberculosis* which was described by Mankiewicz, Stackiewicz and Liivak (79)

Experiments with fungal organisms, pathogenic or not injected into mice infected with *C albicans* led to similar results. A single or repeated administration of *Saccharomyces cerevisiae* to mice after they had been infected with *C albicans* or a simultaneous injection of both organisms led to an increased dissemination of *Candida*. The life of mice injected with *Histoplasma capsulatum* *Cryptococcus neoformans* *Coccidioides immitis* or *Aspergillus fumigatus* 6 days before challenge with *C albicans* was somewhat prolonged It is possible that the modification of the *Candida* infection may have been due to the presence in the mentioned fungi, of endotoxin-like substances.

(ii) (b) *Effects of Living Candida on Nonspecific Resistance to a Candida Infection*

Ninni and Fittipaldi (96) used a pathogenic species of *Candida* which they called *Candida Ninni-Fittipaldi*. They noted that guinea pigs injected intraperitoneally with one large dose of living cells of this *Candida* containing about 1 billion cells do not die. When they are injected, however with four small doses of 50 000 000 cells every four days, about one third of them die in 1—2 days after the fourth injection. There were no survivors after the eighth injection among 12 guinea pigs given 50 000 000 cells intraperitoneally every other day When injected intraperitoneally with 50,000,000 cells every 4—5 days four times and challenged with 500 000 000 cells 5—10 days after the last injection 7 of 8 guinea pigs died 1—3 days after challenge.

The experiments in which guinea pigs died after several intraperitoneal doses of *Candida* the total of which would be sublethal if given in one injection, may be based on liberation of the endotoxin-like substance from living *Candida* which stimulates the growth of the fungus in every subsequent injection as long as the infection has not subsided and the susceptible tissues are still being supplied with enough of this substance The experiment in which most animals died after they had been injected 4 times with a small dose and reinjected 5—10 days after the last injection may be due to the same mechanism or possibly due to sensitization.

Ninni and Fittipaldi have also shown in another experiment which is reviewed in the chapter on specific immunity (pages 37—38) that all guinea pigs receiving two intraperitoneal injections of 1 000 000 000 cells each given 10 days apart died while only 2/3 of them succumbed when the interval was 30

the development of specific antibodies.

Another experiment showed that the llf of mice was prolonged when an injection of *C albicans* or another species of *Candida* was followed 6 days later by a challenge with *C albicans*. A high concentration of the endotoxin-like substance may have been present in the tissues several days before challenge so that the resulting effect was an increase in resistance

(B) Allergy and its Relation to Immunity in Moniliasis

The study of the relation between allergy and susceptibility or resistance to *Candida* infections is of interest because of basic considerations concerning the pathogenesis of *Candida* infections (100) particularly in view of the experimental findings that suggest the possibility that allergy may be one of the mechanisms for the establishment of disseminated moniliasis. The question of this relationship is also important because allergic reactions may interfere with the ultimate outcome of immunization since the antigen used for immunization may also contain an allergen. The elimination of such an allergen may thus lead to the production of a more effective vaccine.

( ) *Att mpt to Prod Bron hopul mon ry Moniliasis without Sensitization*

Lim, Kurot hkin and Wu (76) studied the question whether bronchopulmonary moniliasis may be produced in healthy animals by inoculations of the fungus intratracheally or what factors may be necessary for its establishment. They were unable to produce bronchopulmonary moniliasis in rabbits by intratracheal injections of *Monilia tropicalis* given twice at three day intervals. They were equally unable to produce the disease when direct mechanical injury to the lung was brought about by the injection of either glass powder char coal or phenol and was followed by intra tracheal inoculation of *Monilia tropicalis*. Intravenous injections of small doses of liquid paraffin, though causing the formation of small embolic foci in the lungs, did not enable the *Monilia tropicalis* to develop active lesions. Intravenous injections of chaulmoogra oil produced stable embolic lesions in which *Monilia tropicalis* injected through the trachea developed and gave rise to localized pulmonary or generalized infection.

Reimann and Kurotchkin (108) were unable to produce any pulmonary lesions in monkeys by similar methods as those used in the mentioned experiments in rabbits. Intravenous administration of chaulmoogra oil in monkeys did not predispose to the formation of monilial lesions.

(b) *Production of A tive and Passive Anaphylaxis with Candida or its Prod cts*

In the next series of experiments Kurotchkin and Lim (66) tried to solve the question whether allergic sensitization may be produced by *Candida*. They injected guinea pigs intraperitoneally with heat-killed cells of *Monilia pulonis*, *M Pinoyi* and *Saccharomyces cerevisiae*. Each dose was equal to 1/10 of an agar slant culture. The animals received 1 to 3 such injections. At the end of two weeks all guinea pigs were injected with

tion produced by the first injection may have been subsiding by the time the second injection was given. A sufficient amount of the endotoxin like substance may have been present in the susceptible tissues of the mice several days prior to challenge and very little of it at the time of the challenge. As was shown by own experiments with the soluble extract of *C albicans* (Figures 2, 3 4 and 5) and by those of Hasenclever and Mitchell (40) mentioned on page 21 in which dead cells of *Candida* were used as pretreatment the endotoxin-like substance or dead cells injected several days before challenge enhance the resistance to it.

The experiments of Hasenclever and Mitchell (40) with viable cells also suggest that an endotoxin like substance similar to that of *C albicans* is probably present in members of the genus *Candida* other than *albicans* as may also be concluded from the experiments of Rzucidlo et al. (120) which are reviewed on page 22

The results of these experiments which show the effects of variously spaced repeated injections of living cells of *Candida* given within a relatively short time cannot be easily predicted because of the variables inherent in the use of multiplying agents. The size of the inoculum is of major importance here, since the spread of the fungus in the animal the duration and time of maximum liberation of the endotoxin like substance and consequently either enhancement or depression of resistance to challenge largely depend on it.

It appears that these experiments should be evaluated mainly on the basis of two factors, both of which are besides timing of the injections, largely influenced by the size of the inoculum. One of them is the presence or absence of a high level of the endotoxin-like substance in the animal tissues at the time of challenge. If a high level is present the infection caused by the challenge will be enhanced (Figure 9) The other factor is the presence or absence of this substance in the tissues several days before challenge. If present it will enhance resistance to the infection (Figures 2 3 4 and 5) Various combinations of these two factors may naturally cause enhancement depression or no effect on the resistance of the animal.

Four to eight small inoculations of *C albicans* repeated every 2—4 days killed the majority of guinea pigs while one large injection containing more yeast cells than the total of the repeated injections did not. Liberation of the endotoxin-like substance a high level of which may have been present at the time of each subsequent injection may have stimulated the growth of the fungus. The situation may have been similar to that in the own experiment depicted in Figure 9

Two large injections of *C albicans* given ten days apart killed all guinea pigs while two identical large injections given 30 days apart killed only two thirds of them. When the interval was 10 days enough endotoxin-like substance may have been present at the time of challenge to enhance it. When the interval was 30 days the effect of this substance may have disappeared by the time of the second injection and some animals may have been protected due to

the development of specific antibodies.

Another experiment showed that the lif of mice was prolonged when an injection of C albicans or another species of *Candida* was followed 6 days later by a challenge with C albicans A high concentration of the endotoxin-like substance may have been present in the tissues several days before challenge so that the resulting effect was an increase in resistance.

(B) Allergy and Its Relation to Immunity in Moniliasis

The study of the relation between allergy and susceptibil ty or resistance to *Candida* infections is of interest because of basic considerations concerning the pathogenesis of *Candida* infections (100) particularly in view of the experimental findings that suggest the possibility that allergy may be one of the mechanisms for the establishment of disseminated moniliasis. The question of this relationship is also important because allergic reactions may interfere with the ultimate outcome of immunization since the tigen used for immunization may also contain an allergen. The elimination of such an allergen may thus lead to the production of more effective vaccine.

( ) *Attempt t Produc Bron hopulmo ary Moniliasis without Sensitization*

Lim, Kurotchkin and Wu (76) studied the question whether bronchopulmonary moniliasis may be produced in healthy nimals by inoculations of the fungus intratracheally or what factors may be necessary for its establishment. They were unable to produce bronchopulmonary moniliasis in rabbits by intratracheal injections of *Monilia tropicalis* given twice at three day intervals. They were equally unable to produce the disease when direct mechanical injury to the lung was brought about by the injection of either glass powder char coal or phenol and was followed by intra tracheal inoculation of *Monilia tropicalis* Intravenous injections of small doses of liquid paraffin, though causing the formation of small embolic foci in the lungs, did not enable the *Monilia tropicalis* to develop active lesions. Intravenous injections of chaulmoogra oil produced stable embolic lesions in which *Monilia tropicalis* injected through the trachea developed and gave rise to localized pulmonary or generalized infection.

Reimann and Kurotchkin (108) were unable to produce any pulmonary lesions in monkeys by similar methods as those used in the mentioned experiments in rabbits. Intravenous administration of chaulmoogra oil in monkeys did not predispose to the formation of monilial lesions.

(b) *P oduction of Active and Passive An phylaxis with Candida or its Prod cts*

In the next series of experiments Kurotchkin and Lim (66) tried to solve the question whether allergic sensitization may be produced by *Candida*. They injected guinea pigs intraperitoneally with heat-killed cells of *Monilia psilosis* M Pinoyi and *Sac haromyces cerevisiae* Each dose was equal to 1/10 of an gar slant culture. The animals received 1 to 3 such injections. At the end of two weeks all guinea pigs were injected with

tion produced by the first injection may have been subsiding by the time the second injection was given. A sufficient amount of the endotoxin-like substance may have been present in the susceptible tissues of the mice several days *prior* to challenge, and very little of it at the time of the challenge. As was shown by own experiments with the soluble extract of *C albicans* (Figures 2 3 4 and 5) and by those of Hasenclever and Mitchell (40) mentioned on page 21 in which dead cells of *Candida* were used as pre treatment, the endotoxin-like substance or dead cells injected several days *before challenge enhance the resistance* to it.

The experiments of Hasenclever and Mitchell (40) with viable cells also suggest that an endotoxin like substance *similar to that of C albicans is probably* present in members of the genus *Candida* other than *albicans* as may also be concluded from the experiments of Rzucidlo et al (120) which are reviewed on page 22.

The results of these experiments which show the effects of variously spaced repeated injections of living cells of *Candida* given within a relatively *short* time cannot be *easily predicted* because of the variables inherent in the use of multiplying agents. The size of the inoculum is of major importance here, since the spread of the fungus in the *animal,* the duration and time of maximum liberation of the endotoxin-like substance and consequently either enhancement or depression of resistance to challenge largely depend on it.

It appears that these experiments should be evaluated mainly on the basis of two factors, both of which are, besides timing of the injections, largely influenced by the size of the inoculum. One of them is the presence or absence of a high level of the endotoxin-like substance in the animal tissues at the time of challenge. If a high level is present the infection caused by the challenge will be enhanced (Figure 9) The other factor is the presence or absence of this substance in the tissues several days before challenge If present it will enhance resistance to the infection (Figures 2, 3 4 and 5) Various combinations of these two factors may naturally cause enhancement, *depression* or *no effect* on the resistance of the animal.

Four to eight small inoculations of *C albicans* repeated every 2—4 days killed the majority of guinea pigs while *one large injection containing more yeast* cells than the total of the repeated injections did not. Liberation of the endotoxin-like substance a high level of which may have been present at the *time of each subsequent* injection may have stimulated the growth of the fungus. The situation may have been similar to that in the own experiment *depicted in Figure 9*

Two large injections of *C albicans* given ten days apart killed all guinea pigs while two identical large injections given 30 days apart killed only two thirds of them. When the interval was 10 days enough endotoxin-like substance may have been present at the time of *challenge to enhance it.* When the interval was 30 days the effect of this substance may have disappeared by the time of the second injection and some animals may have been protected due to

the development of specific antibodies.

Another experiment showed that the life of mice was prolonged when an injection of *C albicans* or another species of *Candida* was followed 6 days later by a challenge with *C albicans*. A high concentration of the endotoxin-like substance may have been present in the tissues several days before challenge so that the resulting effect was an increase in resistance.

(B) Allergy and Its Relation to Immunity in Moniliasis

The study of the relation between allergy and susceptibility or resistance to *Candida* infections is of interest because of basic considerations concerning the pathogenesis of *Candida* infections (100) particularly in view of the experimental findings that suggest the possibility that allergy may be one of the mechanisms for the establishment of disseminated moniliasis. The question of this relationship is also important because allergic reactions may interfere with the ultimate outcome of immunization since the antigen used for immunization may also contain an allergen. The elimination of such an allergen may thus lead to the production of a more effective vaccine.

(a) *Attempts t Produce Bronchopulmonary Moniliasis without Sensitization*

Lim, Kurotchkin and Wu (76) studied the question whether bronchopulmonary moniliasis may be produced in healthy animals by inoculations of the fungus intratracheally or what factors may be necessary for its establishment. They wer unable to produce bronchopulmonary moniliasis in rabbits by intratracheal injections of *M nil a tropicalis* given twice at three day intervals. They were equally unable to produce the disease when direct mechanical injury to the lung was brought about by the injection of either glass powder char coal or phenol and was followed by intratracheal inoculation of *Monilia tropicalis*. Intravenous injections of small doses of liquid paraffin, though causing the formation of small embolic foci in the lungs, did not enable the *Monilia tropicalis* to develop active lesions. Intravenous injections of chaulmoogra oil produced stable embolic lesions in which *Monilia tropicalis* injected through the trachea developed and gave rise to localized pulmonary or generalized infection.

Reimann and Kurotchkin (108) were unable to produce any pulmonary lesions in monkeys by similar methods as those used in the mentioned experiments in rabbits. Intravenous administration of chaulmoogra oil in monkey did not predispose to the formation of monilial lesions.

(b) *P oduction of Active and Passive Anaphylaxis with Cand da or its P od cts*

In the next series of experiments Kurotchkin and Lim (66) tried to solve the question whether allergic sensitization may be produced by *Candida*. They injected guinea pigs intraperitoneally with heat-killed cells of *Monilia psil sis M Pinoyi* and *Sac haromyces cerevisiae*. Each dose was equal to 1/10 of an agar slant culture. The animals received 1 t 3 such injections. At the end of two weeks all guinea pigs were injected with

0.5 to 4.0 mg of a homologous water soluble substance derived from one of the mentioned fungi. Both *Monilia* cultures produced active sensitization as evidenced by a typical anaphylactic shock following an injection of the *Monilia* extract Sensitization was not produced by injections of heat killed cells of *Saccharomyces* It was also found that heat killed *M psilosis* grown in glucose broth sensitized more readily than the same fungus grown on Sabouraud's agar The authors believed that the sensitizing power of the mycelial growth which was mostly found in broth was distinctly higher than that of the yeast cell growth of the same fungus.

Kurotchkin and Lim (67) found that incubation of cells of *Monilia Pinoyi* with formaldehyde decreased the sensitizing power of the fungus in guinea pigs.

Lim and Kurotchkin (74) have examined the relationship between the production of anaphylaxis and formation of precipitin and complement fixing antibodies in guinea pigs injected with heat killed cultures of *Monilia Pinoyi* and *M psilosis* They found that in the case of *M Pinoyi* sensitization appeared before the precipitin or complement fixing antibodies could be demonstrated, while with *M psilosis* anaphylactic response was produced only in the presence of demonstrable precipitins. They also reported that the precipitin reaction was more sensitive than complement fixation

Lim and Kurotchkin (75) studied the time factor in anaphylactic sensitization with heat killed cultures of *Monilia tropicalis* They noted that fatal anaphylactic shock could be produced in guinea pigs when a soluble carbohydrate from *Monilia* was injected as early as six days after the animals had been sensitized. This period was shorter than that usually observed in serum sensitization. The authors have also found that with smaller or larger than optimum sensitizing doses a longer time (15 days) was required. No anaphylaxis occurred following challenging injections containing either very high or very low amounts of the carbohydrate.

Kesten and Mott (61) used similar methods to study hypersensitivity to water soluble substances prepared by chemical extraction of whole cells of *Monilia albicans M psilosis M parapsilosis Willia anomala* and *Saccharomyces cerevisiae* Injections of 10 mg of the specific substance from *Monilia psilosis* on 10 consecutive days failed to produce an anaphylactic shock in guinea pigs injected 21 days after the last sensitizing injection with 0.25 to 4 mg of the same soluble substance. Active sensitization was successful when guinea pigs were given 10 consecutive daily intraperitoneal injections of 0.2 cc. of a 5 % suspension of heat killed washed cells of *Monilia psilosis* Seven out of eight animals given an intravenous shocking dose of 0.5 to 2.0 mg of the soluble substance 21 days after the last injection developed an anaphylactic shock and two of them died. Two guinea pigs received 0.2 cc. of a 20 % suspension of ground *Monilia psilosis* and one animal was given 0.4 cc. of a 10 % suspension of intact living cells of *M psilosis* as a shocking dose instead of the soluble substance. All three developed an anaphylactic shock and one of them died.

Passive anaphylaxis to the soluble substance of *M. pilosus* was produced by an intravenous injection of 0.5 or 1.0 cc. of serum from a rabbit immunized against *M. pilosus*. Twenty-four hours later each of 8 guinea pigs received 0.01 to 1.0 mg. of the homologous soluble substance intravenously. Six of the eight passively sensitized guinea pigs died in anaphylactic shock, the remaining two survived but showed signs of shock. Animals sensitized with each antiserum were also challenged 24 hours later with soluble substances prepared from each of 4 other aforementioned fungi. The heterologous soluble substances often induced anaphylactic shock in passively sensitized guinea pigs. A close relationship was found between positive precipitin reactions and the production of anaphylaxis.

(c) *Production of Bronchopulmonary Moniliasis in Sensitized Animals*

After they had failed to demonstrate a production of bronchopulmonary moniliasis in healthy animals and in most of those with a mechanical injury to the lung, and after they had produced sensitization to *Candida* as evidenced by anaphylactic shock, Kurotchkin and Lim (68) attempted to produce bronchomoniliasis in normal and passively or actively sensitized rabbits. They injected 5 animals intratracheally on 2 successive occasions, 7 days apart, with 1/5 of an agar culture of *Monilia tropicalis*. Rabbits were killed 2 weeks later and no lesions were found on autopsy. A second group of 5 rabbits received an intratracheal injection of 1 cc. of an anti-*Monilia* serum followed by 2 intratracheal injections of *Monilia tropicalis* 7 days apart. The results were essentially negative except for one rabbit in which a monilial lesion was found in one lung. A third group of 8 rabbits received 3 intravenous injections of heat-killed cultures of *Monilia tropicalis* at 5-day intervals. Ten days after the last injection, when precipitin tests became positive, the rabbits were inoculated intratracheally with *M. tropicalis*. This injection was repeated 7 days later. Two weeks after the second inoculation autopsies revealed that 6 of 8 animals developed localized pulmonary lesions which were nodular, caseous in the center. Predisposing sensitivity was thought to account for the pathologic lesions.

Similar experiments were done by Vogel and Krehl (145) who used a single injection of dead *Candida* and adjuvant or living *Candida* for sensitization and living or dead *Candida* for intratracheal challenge. These authors injected two groups of nine and one group of five guinea pigs subcutaneously with a water-in-oil emulsion containing 0.22 cc. of a "thick saline suspension" of heat-killed *Candida albicans* and an adjuvant containing 3 mg. of autoclaved tubercle bacilli in mineral oil and lanolin. One group of 9 guinea pigs was injected at the same time with a single injection of a "heavy suspension" of living cells of *C. albicans*. Another group of 9 guinea pigs was not pretreated in any way.

Animals in one of the 3 groups which received dead *Candida* with adjuvant, animals of the group which was given living *Candida*, and the guinea pigs without any pretreatment, were given

an intratracheal injection of either 5×10^8 2.3×10^7 or 1.2×10^8 of living cells of *C albicans* 12 days after the pretreatment. The group of 5 animals pretreated with dead *Candida* and adjuvant received a challenge of 5×10^8 of dead cells of *Candida*. The third group pretreated with dead *Candida* and adjuvant received no challenge.

While pretreated guinea pigs, especially those that received dead *Candida* with adjuvant, became highly sensitive to skin test materials, it was impossible to induce a chronic or lethal infection by intratracheal challenge with living organisms. The animals were sacrificed nine days after challenge. Hemorrhagic changes in the lungs and thickening of bronchial walls and alveolar septa seen in guinea pigs pretreated with the dead fungus and adjuvant and challenged with living *Candida* and to a lesser extent in those challenged with dead *Candida* were viewed as an expression of hypersensitivity developing in the lung tissue.

The experiments of the group of Kurotchkin, Lim, Reimann and Wu (66—68 74—76 108) show that bronchopulmonary moniliasis cannot be produced in healthy rabbits or monkeys by intratracheal inoculations of the fungus. These investigators were equally unable to induce bronchopulmonary moniliasis by mechanical injury to the lung followed by intratracheal inoculation of *Candida* They did, however produce pulmonary lesions due to *Candida* in rabbits actively sensitized with heat killed cells of *Candida* They concluded from these experiments that allergy is one of the possible mechanisms for the establishment of disseminated moniliasis.

Kesten and Mott (61) produced hypersensitivity to dead cells of *Candida* but not to a soluble substance of the organism. They also succeeded in sensitizing rabbits passively Vogel and Krehl (145) produced hemorrhagic changes in the lungs and thickening of bronchial walls and alveolar septa by sensitizing guinea pigs. Own experiments demonstrate that it is possible to produce anaphylaxis regularly with a fraction of the organism, viz. the soluble extract prepared by the disintegration of *Candida*, while previous authors were able to produce it with whole cells only The fraction used unsuccessfully by Kesten and Mott for the production of anaphylaxis was prepared by chemical extraction of whole intact cells of *Candida* so that it appears that it contained predominantly material originating on or close to the cell surface, or that at least the content of materials of cytoplasmic origin was much lower than in the own soluble extract which was prepared by disintegration of the yeast cells and contained mainly the cytoplasm, besides, possibly the slimy layer covering the outer surface of the cell wall, and some soluble constituents of the cell walls (pages 53—54) It was impossible under my experimental conditions, to produce anaphylaxis in guinea pigs using cell walls for sensitization. All these facts point to the assumption that most of the sensitizing properties reside in the cytoplasm.

It appears, therefore, that since the soluble extract used in my experiments does not contain a specific protective

antigen, since it contains a highly sensitizing substance and since it also contains the endotoxin-like substance capable, under certain conditions, of counteracting the effect of specific immunization, it should not be a part of a protective vaccine against *Candida*.

(C) Specific Immunity

(1) *Active Immunization*

Among the various experiments with the aim of inducing immunity against moniliasis in animals the attempts at active immunization by the introduction of variously prepared antigens are the most numerous. It is impossible to evaluate a great many of them since some reports do not contain enough information concerning the number of organisms used, the timing of injections and the interval between the last vaccine injection and challenge.

(a) *Active Immunization with Living Cells in Rabbits*

Rabbits were used by Ostrowsky (99) who reported preliminary results of his experiments which indicated that increased resistance could be induced by repeated subcutaneous injections of the living but attenuated fungus.

Roger (111 and 112) was able to immunize rabbits by injecting intravenously increasing doses of an *Oidium albicans* culture of low virulence. These rabbits were able to survive a challenge dose of two to three lethal doses. Details concerning the number of organisms or timing of injections were not given.

Roger also observed abundant growth of *Oidium albicans* in normal rabbit serum as compared to a very marked inhibition of growth in the serum of immunized animals. He noticed agglutination of *Candida* cells in the serum of immunized rabbits and the presence of a thick hyaline membrane surrounding the yeast cells. This membrane was sometimes lightly striated, with poorly defined borders. Its thickness was 5 to 10 times that of a normal cell wall.

Charrin and Ostrowsky (11) reported immunization of rabbits with repeated intravenous injections of a living but attenuated *Oidium albicans*. The fungus did not grow well in the serum of immunized animals. Details of the experiments are not given.

Concetti (14) reported that rabbits pretreated with repeated increasing intravenous doses of cultures of *Oidium albicans* subjected to slow desiccation for 1 or 2 weeks survived an intravenous challenge with a number of organisms much higher than the lethal dose. Neither the exact number of organisms used for immunization or challenge nor the timing of the injections were given.

van der Linden (143) administered to rabbits six subcutaneous injections of increasing amounts of live or heat-killed organisms of *Monilia albicans*. The injections were given every eight days. After an unspecified length of time the animals were challenged with an intravenous injection of 2.5 times the lethal dose for the same organism. All rabbits died in less than four days presenting typical symptoms of generalized moniliasis.

The information given in all of the

an intratracheal injection of either 5×10^5, 2.3×10^7 or 1.2×10^8 of living cells of *C. albicans* 12 days after the pretreatment. The group of 5 animals pretreated with dead *Candida* and adjuvant received a challenge of 5×10^8 of dead cells of *Candida*. The third group pretreated with dead *Candida* and adjuvant received no challenge.

While pretreated guinea pigs, especially those that received dead *Candida* with adjuvant, became highly sensitive to skin test materials, it was impossible to induce a chronic or lethal infection by intratracheal challenge with living organisms. The animals were sacrificed nine days after challenge. Hemorrhagic changes in the lungs and thickening of bronchial walls and alveolar septa seen in guinea pigs pretreated with the dead fungus and adjuvant and challenged with living *Candida*, and to a lesser extent in those challenged with dead *Candida* were viewed as an expression of hypersensitivity developing in the lung tissue.

The experiments of the group of Kurotchkin, Lim, Reimann and Wu (66—68, 74—76, 108) show that bronchopulmonary moniliasis cannot be produced in healthy rabbits or monkeys by intratracheal inoculations of the fungus. These investigators were equally unable to induce bronchopulmonary moniliasis by mechanical injury to the lung followed by intratracheal inoculation of *Candida*. They did, however, produce pulmonary lesions due to *Candida* in rabbits actively sensitized with heat-killed cells of *Candida*. They concluded from these experiments that allergy is one of the possible mechanisms for the establishment of disseminated moniliasis.

Kesten and Mott (61) produced hypersensitivity to dead cells of *Candida* but not to a soluble substance of the organism. They also succeeded in sensitizing rabbits passively. Vogel and Krehl (145) produced hemorrhagic changes in the lungs and thickening of bronchial walls and alveolar septa by sensitizing guinea pigs. Own experiments demonstrate that it is possible to produce anaphylaxis regularly with a fraction of the organism, viz. the soluble extract prepared by the disintegration of *Candida*, while previous authors were able to produce it with whole cells only. The fraction used unsuccessfully by Kesten and Mott for the production of anaphylaxis was prepared by chemical extraction of whole intact cells of *Candida* so that it appears that it contained predominantly material originating on or close to the cell surface, or that at least the content of materials of cytoplasmic origin was much lower than in the own soluble extract which was prepared by disintegration of the yeast cells and contained mainly the cytoplasm, besides, possibly, the slimy layer covering the outer surface of the cell wall, and some soluble constituents of the cell walls (pages 53—54). It was impossible under my experimental conditions, to produce anaphylaxis in guinea pigs using cell walls for sensitization. All these facts point to the assumption that most of the sensitizing properties reside in the cytoplasm.

It appears, therefore, that since the soluble extract used in my experiments does not contain a specific protective

antigen, since it contains a highly sensitizing substance and since it also contains the endotoxin-like substance capable, under certain conditions, of counteracting the effect of specific immunization, it should not be a part of a protective vaccine against *Candida*.

(C) Specific Immunity

(1) *Active Immunization*

Among the various experiments with the aim of inducing immunity against moniliasis in animals the attempts at active immunization by the introduction of variously prepared antigens are the most numerous. It is impossible to evaluate a great many of them since some reports do not contain enough information concerning the number of organisms used, the timing of injections and the interval between the last vaccine injection and challenge.

( ) *Active Immunization with Living Cells in Rabbits*

Rabbits were used by Ostrovsky (99) who reported preliminary results of his experiments which indicated that increased resistance could be induced by repeated subcutaneous injections of the living but attenuated fungus.

Roger (111 and 112) was able to immunize rabbits by injecting intravenously increasing doses of an *Oidium albicans* culture of low virulence. These rabbits were able to survive a challenge dose of two to three lethal doses. Details concerning the number of organisms or timing of injections were not given.

Roger also observed abundant growth of *Oidium albicans* in normal rabbit serum as compared to a very marked inhibition of growth in the serum of immunized animals. He noticed agglutination of *Candida* cells in the serum of immunized rabbits and the presence of a thick hyaline membrane surrounding the yeast cells. This membrane was sometimes lightly striated, with poorly defined borders. Its thickness was 5 to 10 times that of a normal cell wall.

Charrin and Ostrowsky (11) reported immunization of rabbits with repeated intravenous injections *of a living but attenuated Oidium albicans*. The fungus did not grow well in the serum of immunized animals. Details of the experiments are not given.

Concetti (14) reported that rabbits pretreated with repeated increasing intravenous doses of cultures of *Oidium albicans subjected to slow* desiccation for 1 or 2 weeks survived an intravenous challenge with a number of organisms much higher than the lethal dose. Neither the exact number of organisms used for immunization or challenge nor the timing of the injections were given.

van der Linden (143) administered to rabbits six subcutaneous injections of increasing amounts of live or heat-killed organisms of *Monilia albicans*. The injections were given every eight days. After an unspecified length of time the animals were challenged with an intravenous injection of 2.5 times the lethal dose for the same organism. All rabbits died in less than four days presenting typical symptoms of generalized moniliasis.

The information given in all of the

above mentioned papers is not sufficient to permit their adequate evaluation.

Cavallero (10) used three different schedules to immunize rabbits with living cells of *Mycotorula albicans*

The first group was pretreated with a single intravenous injection of 2 billion blastospores and challenged 10 to 15 days later intravenously with a massive dose (presumably 2 billion blastospores) of a strain of the fungus said to be more pathogenic than the one used for pre treatment.

The second group was given 3 intra venous injections of 1 2 and 2 billion blastospores, respectively of the same strain that was used for the pretreatment in the first group The second and third injection was given 4 or 5 and 10 or 11 days, respectively after the first one. These rabbits were challenged with 150 million cells of the more pathogenic strain, which was twice its minimum lethal dose, 30 to 45 days after the last injection.

The third group of rabbits was injected either intravenously or subcutaneously with 500 000 1,000,000 and 10 000 000 cells of the more pathogenic strain and challenged 30 to 45 days after *the last injection with 150 million* cells of the same strain. The timing of *the immunizing injections was the same* as in the preceding group

Except for an insignificant number of rabbits in the first group all animals died at about the same time as the untreated controls.

The author noticed that mycotic lesions in pretreated animals had a more hemorrhagic and exudative character than those of control animals. He concluded that this may be due to a mechanism similar to the Shwartzman phenomenon, and, possibly due to increased susceptibility to infection in pre treated animals. He speculated that some of the rabbits that survived for a longer period of time may have been slightly protected. The author did not exclude the possibility that both increased susceptibility and protection may have been the result of immunization in his experiments.

It is possible that Cavallero (10) may have rendered the rabbits hypersensitive. This is probable especially since he *found that their lesions had a more* hemorrhagic and exudative character than those of rabbits which were not pretreated. Similar pathologic lesions were observed in sensitized guinea pigs by Vogel and Krehl (145)

Liberation of the endotoxin-like substance from living *Candida* cells may also have been taking place. This substance may have stimulated the growth of *Candida* and its effect may have decreased the protective action of the specific cell wall antigen. It is also possible that the number of cells in the vaccine injections was insufficient

Hoffmeister Dickgiesser and Gotting (48) applied a combined schedule using first heat killed and later living cells of *Candida albicans* They immunized 4 rabbits first with heat killed cells, starting with 2 subcutaneous injections of 500 000 cells each on the first and fourth day injecting intravenously 5,000 000 cells on the seventh day and 10 000,000 cells on the eleventh day In the further course of immunization living cells were used by intravenous route. The animals were

given 10,000,000 cells on the 18th day and 5 billion cells on the 34th day. Six days later they received an intravenous challenge dose containing 5 billion cells. A nonimmunized control rabbit which was given the same challenge dose died 5 days later with symptoms of generalized moniliasis. Two of the four immunized rabbits were killed 11 days after challenge and revealed no signs of moniliasis on autopsy. The fate of the remaining two immunized rabbits was not reported.

In these apparently successful experiments only dead *Candida* cells were used in the first four injections. This eliminated a source of the endotoxin-like substance which would have been constantly liberated from a living antigen capable of multiplication. The living cells contained in successive injections could not, therefore, be stimulated by an uncontrollable supply of the endotoxin-like substance. Quite the opposite may have occurred when the fifth immunizing injection, which contained living cells, was given 7 days after the fourth one, on the 18th day of treatment. The endotoxin-like substance from the preceding injection may have been effective in suppressing the fungus growth by the mechanism of nonspecific immunity as demonstrated by own experiments (Figures 2, 3, 4 and 5). By that time specific antibodies may have developed and been instrumental in increasing resistance to the remaining two intravenous injections of living cells.

Wunner (151) immunized rabbits intravenously by either single injection of sublethal number of living *Candida* cells or by repeated intravenous injections of formol-killed cells. The frequency of injections or the number of organisms were not given. After an unspecified length of time the rabbits were challenged with an intravenous injection of 10 to 100 million living cells of *Candida albicans*. Eighteen immunized rabbits, two of which possessed natural agglutinins, and 18 nonimmunized controls died in from 1 to 6 days. It was not stated how many rabbits were immunized with living and how many with dead cells. One immunized rabbit survived. Immunized animals had slightly to moderately elevated agglutinin titres. The lesions produced in immunized rabbits resembled those noted in the nonimmunized group. The author concluded that agglutinins did not protect the animals from the lethal effects of the organism. He believed that *Candida* elaborated a toxin which was unaffected by agglutinins.

It is impossible to determine the reasons for the poor results of immunization because of insufficient information.

(b) *Active Immunization with Living Cells in Guinea Pigs*

Concetti (14) was unable to protect guinea pigs against challenge by injections of increasing doses of cultures of *Oidium albicans* attenuated by slow desiccation for 1 or weeks. The injections were given over a period of several weeks every or 5 days subcutaneously or intraperitoneally. Too few data were presented by the author to permit any evaluation of the experiment.

Nimi and Fitzpaldi (96) used for their experiments a pathogenic *Candida* which they called *Candida Nimi-*

Fittipaldi (page 28) They noted that two intraperitoneal injections of 1 000 000 000 cells each given 10 days apart kill all animals 1 to 3 days after the second injection. When reinjected in 30 instead of 10 days about 2/3 of the animals died.

In another experiment guinea pigs were injected either with one intraperitoneal injection containing 500 000 000 cells of *Candida* or with four intraperitoneal doses of 50 000 000 cells each and challenged ten or thirty days after the last injection with 100 000 000 cells administered intraperitoneally. The animals were killed 1/2 hour or 3 hours after challenge. Fungi could be found anywhere scattered in the peritoneal cavity in controls which received the challenge dose only while pretreated animals had organisms only in the epiploon. Hemorrhagic exudate was present in the abdominal cavity of pretreated animals while there was none in controls receiving the challenge dose only.

Since the guinea pigs in these experiments of Ninni and Fittipaldi receiving 1 000 000 000 cells did not die within 30 days following the first injection it appears that the infection may have been subsiding. The number of living cells was probably not sufficient to liberate enough endotoxin like substance in the susceptible tissues of these guinea pigs at the time of the second injection to enhance the infection sufficiently as to produce death of all animals. Hypersensitivity, an insuffient level of specific antibodies, as well as some effect of the endotoxin-like substance on the depression of nonspecific resistance may have been responsible for the deaths of two thirds of animals which did occur in this group.

In the experiment in which the interval between the two injections was ten days, more endotoxin-like substance may have been present at the time of the second injection. Another factor playing a role in the death of all animals reinjected after 10 days may have been the possibility that this interval was too short for the development of specific immunity.

(c) *Active Immunization with Living Cells in Mice*

Fischer and Horbach (31) have injected *60 mice intraperitoneally with 0.2 cc. of* a 4 % suspension of *Candida albicans* (which in own experience contains about 100 000 000 cells). On the 23rd day 33 surviving mice were challenged intraperitoneally with an equal dose of *Candida*. Three weeks later 28 mice were dead while only 19 of 33 control mice died in the same period of time. The authors concluded that specific infection immunity to *Candida* did not exist in mice.

The endotoxin like substance contained in the first dose may have enhanced the growth of organisms in the second dose and caused higher death rate. The role of allergic sensitization in the enhancement of the *Candida* infection may also be considered.

Mourad and Friedman (89) treated mice with 6 subcutaneous injections of vaccines given 40, 30, 20, 12, 5 and 1 day before intravenous challenge with 10^4 or 10^5 viable cells of *Candida albicans*. Three different vaccines were

used viable cells, merthiolate-killed cells and cells vibrated for nearly 7 hours in a sonic oscillator. A single dose of each vaccine corresponded to 40,000,000 cells. Thirty to forty mice were used in each group. All 49 nonvaccinated controls challenged with 100,000 cells died in less than 35 days. One hundred and forty days after challenge the mortality rates were 50 %, 73 % and 77 % respectively for mice treated with sonically vibrated, merthiolate-killed and living vaccines. A total of 62 % of 45 nonimmunized controls which received an intravenous challenge of 10,000 cells were dead 140 days after they had been infected. The mortality rates of immunized mice in this experiment were 17 %, 36 % and 45 % respectively for animals pretreated with sonically vibrated, merthiolate-killed and living cells. All mice, treated and untreated, had lesions caused by *Candida* indistinguishable from each other.

Among the three groups better results were obtained with sonically vibrated and merthiolate-killed cells than with the viable vaccine. This may have been caused by the fact that the endotoxin-like substance contained in each subsequent injection could not have enhanced the growth of cells in the preceding injections, since they were dead. It is possible that some of the endotoxin-like substance of *Candida* may have been released by sonic vibrations and may have been more easily available from this preparation than from intact live or merthiolate-killed cells. Although the vaccine was injected subcutaneously, by route in which endotoxins apparently influence nonspecific resistance to a relatively slight extent (62 and Figure 10), it is possible that the endotoxin-like substance may have increased nonspecific resistance to the intravenous challenge especially since an injection was given also 5 days before challenge, which is usually the time of about the maximum nonspecific enhancement of resistance.

Undoubtedly specific immunity played an important role here and the question is tempting whether a more pronounced protection may not have been obtained by the treatment of the cells with the vibrator by which some of them may have been ruptured and the specific antigen may have become more easily available. An easier availability could be explained on the assumption that the protective antigen may be located in an inside layer of the cell walls which may have become exposed to the tissues of the host after mechanical disintegration of the cells.

A relative success of the immunization with living cells which, as all other vaccines of Mourad and Friedman, were injected subcutaneously as compared to the experiments of others who have utilized the intraperitoneal or intravenous route may be explained by the fact that living cells injected into the subcutaneous space could not easily spread and cause generalized moniliasis. Moreover as pointed out before, the endotoxin is only slightly effective in its action on nonspecific resistance when given subcutaneously. Furthermore the endotoxin-like substance is obviously less easily available from viable intact cells than from a vaccine prepared by sonic vibration.

Fittipaldi (page 28) They noted that two intraperitoneal injections of 1 000 000 000 cells each given 10 days apart kill all animals 1 to 3 days after the second injection. When reinjected in 30 instead of 10 days about 2/3 of the animals died.

In another experiment guinea pigs were injected either with one intraperitoneal injection containing 500 000 000 cells of *Candida* or with four intraperitoneal doses of 50 000 000 cells each and challenged ten or thirty days after the last injection with 100 000 000 cells administered intraperitoneally The animals were killed 1/2 hour or 3 hours after challenge. Fungi could be found anywhere scattered in the peritoneal cavity in controls which received the challenge dose only while pretreated animals had organisms only in the epiploon. Hemorrhagic exudate was present in the abdominal cavity of pretreated animals while there was none in controls receiving the challenge dose only

Since the guinea pigs in these experiments of Ninni and Fittipaldi receiving 1 000 000 000 cells did not die within 30 days following the first injection it appears that the infection may have been subsiding The number of living cells was probably not sufficient to liberate enough endotoxin like substance in the susceptible tissues of these guinea pigs at the time of the second injection to enhance the infection sufficiently as to produce death of all animals. Hypersensitivity an insuffient level of specific antibodies, as well as some effect of the endotoxin-like substance on the depression of nonspecific resistance may have been responsible for the deaths of two thirds of animals which did occur in this group

In the experiment in which the interval between the two injections was ten days, more endotoxin-like substance may have been present at the time of the second injection. Another factor playing a role in the death of all animals reinjected after 10 days may have been the possibility that this interval was too short for the development of specific immunity

(c) *Active Immunization with Living Cells in Mice*

Fischer and Horbach (31) have injected 60 mice intraperitoneally with 0.2 cc. of a 4 % suspension of *Candida albicans* (which in own experience contains about 100 000 000 cells) On the 23rd day 33 surviving mice were challenged intraperitoneally with an equal dose of *Candida.* Three weeks later 28 mice were dead while only 19 of 33 control mice died in the same period of time. The authors concluded that specific infection immunity to *Candida* did not exist in mice.

The endotoxin-like substance contained in the first dose may have enhanced the growth of organisms in the second dose and caused higher death rate. The role of allergic sensitization in the enhancement of the *Candida* infection may also be considered.

Mourad and Friedman (89) treated mice with 6 subcutaneous injections of vaccines given 40 30 20 12 5 and 1 day before intravenous challenge with 10^6 or 10^7 viable cells of *Candida albicans.* Three different vaccines were

used viable cells, merthiolate-killed cells and cells vibrated for nearly 7 hours in a sonic oscillator. A single dose of each vaccine corresponded to 40,000,000 cells. Thirty to forty mice were used in each group. All 49 nonvaccinated controls challenged with 100,000 cells died in less than 55 days. One hundred and forty days after challenge the mortality rates were 50 %, 73 % and 77 % respectively for mice treated with sonically vibrated, merthiolate-killed and living vaccines. A total of 62 % of 45 nonimmunized controls which received an intravenous challenge of 10,000 cells were dead 140 days after they had been infected. The mortality rates of immunized mice in this experiment were 17 %, 36 % and 43 % respectively for animals pretreated with sonically vibrated, merthiolate-killed and living cells. All mice, treated and untreated, had lesions caused by *Candida* indistinguishable from each other.

Among the three groups better results were obtained with sonically vibrated and merthiolate-killed cells than with the viable vaccine. This may have been caused by the fact that the endotoxin-like substance contained in each subsequent injection could not have enhanced the growth of cells in the preceding injections, since they were dead. It is possible that some of the endotoxin-like substance of *Candida* may have been released by sonic vibrations and may have been more easily available from this preparation than from intact live or merthiolate-killed cells. Although the vaccine was injected subcutaneously by a route in which endotoxins apparently influence nonspecific resistance to a relatively slight extent (62 and Figure 10), it is possible that the endotoxin-like substance may have increased nonspecific resistance to the intravenous challenge, especially since an injection was given also 5 days before challenge which is usually the time of about the maximum nonspecific enhancement of resistance.

Undoubtedly specific immunity played an important role here and the question is tempting whether a more pronounced protection may not have been obtained by the treatment of the cells with the vibrator by which some of them may have been ruptured and the specific antigen may have become more easily available. An easier availability could be explained on the assumption that the protective antigen may be located in an inside layer of the cell walls which may have become exposed to the tissues of the host after mechanical disintegration of the cells.

A relative success of the immunization with living cells which, as all other vaccines of Mourad and Friedman, were injected subcutaneously as compared to the experiments of others who have utilized the intraperitoneal or intravenous route may be explained by the fact that living cells injected into the subcutaneous space could not easily spread and cause generalized moniliasis. Moreover as pointed out before, the endotoxin is only slightly effective in its action on nonspecific resistance when given subcutaneously. Furthermore, the endotoxin-like substance is obviously less easily available from viable intact cells than from a vaccine prepared by sonic vibration.

In the case of the merthiolate treated vaccine with which the results were slightly better than with the live vaccine there were no living cells in the immunizing injections to be enhanced by the nonspecific mechanism.

(d) *Active Immunization with Dead Cells in Rabbits*

Some reports are also available on active immunization of animals with dead cells. Ostrovsky (99) reported that a rabbit repeatedly inoculated by the subcutaneous route over a period of one and a half months with 50 cc. of heat killed cells did not survive a challenge of 2 cc. of a virulent culture. His serum was not fungistatic. The number of cells, number and frequency of injections or the interval between the last injection and the challenge was not given. Another rabbit similarly treated did not survive the challenge

Connor (15) immunized a rabbit with 41 cc. of a suspension of heat killed cells of *Monilia psilosis* divided into 6 doses over a period of 6 weeks. The number of organisms contained in this vaccine was not given The animal was challenged after an unspecified length of time with an intravenous injection of living cells and died in 4 days, the same length of time as a nonimmunized control. His lesions were less extensive than those of the control rabbit. The author concluded that this was an expression of a slight relative immunity

The unsuccessful immunization experiments of van der Linden (143) in which the author used also heat killed *Monilia albicans* besides a viable vaccine have been mentioned in the section dealing with immunization with viable cells (page 35) The injection schedules and the results were the same for live and dead cells.

The experiments of the three preceding authors cannot be adequately evaluated because of lack of specific data.

Cavallero (10) whose experiments were also mentioned in the preceding section (page 36) used heat killed cells besides live ones for immunization He injected rabbits intravenously with 25 50 and 150 million heat killed cells of a strain of *Mycotorula albicans* which had a high pathogenicity The second and third injections were given 4 or 5 and 10 or 11 days, respectively after the first one. The animals were challenged with 150 million living cells of the homologous strain which was twice its minimum lethal dose 30 to 45 days after the last vaccine injection. All animals died at about the same time as the untreated controls. There was no difference in the results between rabbits immunized with living or heat killed cells.

Because of the long interval between the last immunizing injection and challenge (30 to 45 days) nonspecific immunity was apparently not playing any role in these experiments. The factors responsible for the negative results may have been allergic sensitization and, possibly insufficient numbers of cells contained in the vaccine injections.

Hurd and Drake (53) immunized actively 2 rabbits by treating them intravenously for almost 9 months with an unspecified number of injections of heat killed cells of *Candida albicans* preserved with formalin. Six days after the last injection they received a chal

lenge dose of *C. albicans*. Both died in less than 48 hours. The authors concluded that active immunization was ineffective and possibly deleterious.

The immunization experiments of Winner (151) with a single intravenous injection of living organisms have been mentioned on page 37. The author used also repeated intravenous injections of formol-killed cells of *Candida albicans* and concluded that immunization did not protect rabbits against fatal challenge.

Neither the experiments of Hurd and Drake nor those of Winner may be evaluated because of lack of data. It is possible that the rabbits of Hurd and Drake may have become hypersensitive.

( ) *Active Imm nization with Dead Cells n Guine Pigs*

Mazzetti and Fisu Marracelni (83) ha e immunized guinea pigs by injecting them subcutaneously with 250 and .00 million or 250 500 and 1,000 million formalin-killed cells of *Candida albicans* or *C pseudotropicalis* given t intervals of 7 days. Twelve days after the last injection all nimals were given an intravenous challenge of living cultur of *C alb ans* which killed controls between 7 and 14 days. The number of animals used or the size of challenge dose was *not stated* except that it was a 1 250 dilution of 24 hour agar cultur) At the time of death of the last control animal all immunized guinea pigs were live. On utopsy ll organs were negative for fungi on culture except for one nimal immunized with two doses of *C pse dotropicalis*.

These experiments may have been successful because no living cells were used which may have been enhanced by the endotoxin-like substance contained in the preceding injections. If the conclusions of Kurotchkin and Lim (67) are correct, the guinea pigs in this experiment may not have been strongly sensitized since formalin was used to kill the cells. The number of cells used for immunization was higher and therefore apparently more adequate than in most other experiments. It is possible that since the challenge dose was given 12 days after the last immunizing injection the nonspecific effect of the endotoxin may still have been operative in increasing resistance.

(f) *Active Immunization with Dead C lls in Mice*

Marcus and Rambo (80) injected mice intraperitoneally once weekly for 3 weeks with formalin-killed suspensions of yeast cells of *Candida albicans*. The mice were challenged after an unspecified length of time intravenously with one or more LD_{50} of the living organism. There was no significant difference between the mortality rates of immunized and control animals but kidney cultures of immunized mice were usually negative unlike those of control animals.

Hill (46) immunized mice with formalin-killed cells of *Candida albicans* injected *intraperitoneally and found* slightly lower mortality *rates than in* controls. Organs of immunized animals contained fewer viable organisms than those of nonimmunized *controls*.

Because of insufficient information a critical appraisal of these experiments is impossible.

In the case of the merthiolate-treated vaccine with which the results were slightly better than with the live vaccine there were no living cells in the immunizing injections to be enhanced by the nonspecific mechanism.

(d) *Active Immunization with Dead Cells in Rabbits*

Some reports are also available on active immunization of animals with dead cells. Ostrovsky (99) reported that a rabbit repeatedly inoculated by the subcutaneous route over a period of one and a half months with 50 cc. of heat killed cells did not survive a challenge of 2 cc. of a virulent culture. His serum was not fungistatic. The number of cells, number and frequency of injections or the interval between the last injection and the challenge was not given. Another rabbit similarly treated did not survive the challenge.

Connor (15) immunized a rabbit with 41 cc. of a suspension of heat killed cells of *Monilia psilosis* divided into 6 doses over a period of 6 weeks. The number of organisms contained in this vaccine was not given. The animal was challenged after an unspecified length of time with an intravenous injection of living cells and died in 4 days, the same length of time as a nonimmunized control. His lesions were less extensive than those of the control rabbit. The author concluded that this was an expression of a slight relative immunity.

The unsuccessful immunization experiments of van der Linden (143) in which the author used also heat killed *Monilia albicans* besides a viable vaccine have been mentioned in the section dealing with immunization with viable cells (page 35). The injection schedules and the results were the same for live and dead cells.

The experiments of the three preceding authors cannot be adequately evaluated because of lack of specific data.

Cavallero (10) whose experiments were also mentioned in the preceding section (page 36) used heat killed cells besides live ones for immunization. He injected rabbits intravenously with 25, 50 and 150 million heat killed cells of a strain of *Mycotorula albicans* which had a high pathogenicity. The second and third injections were given 4 or 5 and 10 or 11 days, respectively after the first one. The animals were challenged with 150 million living cells of the homologous strain which was twice its minimum lethal dose, 30 to 45 days after the last vaccine injection. All animals died at about the same time as the untreated controls. There was no difference in the results between rabbits immunized with living or heat-killed cells.

Because of the long interval between the last immunizing injection and challenge (30 to 45 days) nonspecific immunity was apparently not playing any role in these experiments. The factors responsible for the negative results may have been allergic sensitization and, possibly, insufficient numbers of cells contained in the vaccine injections.

Hurd and Drake (53) immunized actively 2 rabbits by treating them intravenously for almost 9 months with an unspecified number of injection of heat killed cells of *Candida albicans* preserved with formalin. Six days after the last injection they received a chal

lenge dose of *C. albicans*. Both died in less than 48 hours. The authors concluded that active immunization was ineffective and possibly deleterious.

The immunization experiments of Winner (151) with single intravenous injection of living organisms have been mentioned on page 37. The author used also repeated intravenous injections of formol-killed cells of *Candida albicans* and concluded that immunization did not protect rabbits against fatal challenge.

Neither the experiments of Hurd and Drake nor those of Winner may be evaluated because of lack of data. It is possible that the rabbits of Hurd and Drake may have become hypersensitive.

(e) *Active Immunization with Dead Cells in Guinea Pigs*

Mazzetti and Fiori Marracconi (83) have immunized guinea pigs by injecting them subcutaneously with 250 and 500 million or 250, 500 and 1,000 million formalin-killed cells of *Candida albicans* or *C. pseudotropicalis* given at intervals of 7 days. Twelve days after the last injection all animals were given an intravenous challenge of a living culture of *C. albicans* which killed controls between 7 and 14 days. The number of animals used or the size of challenge dose was not stated except that it was a 1:250 dilution of a 24 hour agar culture). At the time of death of the last control animal all immunized guinea pigs were alive. On autopsy all organs were negative for fungi on culture except for one animal immunized with two doses of *C. pseudotropicalis*.

These experiments may have been successful because no living cells were used which may have been enhanced by the endotoxin-like substance contained in the preceding injections. If the conclusions of Kurotchkin and Lim (67) are correct, the guinea pigs in this experiment may not have been strongly sensitized since formalin was used to kill the cells. The number of cells used for immunization was higher and therefore apparently more adequate than in most other experiments. It is possible that since the challenge dose was given 12 days after the last immunizing injection the nonspecific effect of the endotoxin may still have been operative in increasing resistance.

(f) *Active Immunization with Dead Cells in Mice*

Marcus and Rambo (80) injected mice intraperitoneally once weekly for 3 weeks with formalin-killed suspensions of yeast cells of *Candida albicans*. The mice were challenged after an unspecified length of time intravenously with one or more LD_{50} of the living organism. There was no significant difference between the mortality rates of immunized and control animals but kidney cultures of immunized mice were usually negative unlike those of control animals.

Hill (46) immunized mice with formalin-killed cells of *Candida albicans* injected intraperitoneally and found slightly lower mortality rates than in controls. Organs of immunized animals contained fewer viable organisms than those of nonimmunized controls.

Because of insufficient information a critical appraisal of these experiments is impossible.

The experiments of Mourad and Friedman (89) have been discussed in the section on immunization with living cells (pages 38—40) As was pointed out there, mortality rates of mice immunized with merthiolate-killed and sonically vibrated cells were lower than of those immunized with living cells.

(g) *Active Immunization with Fractions or Products of Candida Cells*

Attempts have been made to produce active specific immunity in animals by the use of fractions of cells of *Candida* or their cellular products.

Ostrovsky (99) reported that a single subcutaneous injection of 20 cc. of a culture filtrate of *Oidium albicans* did not immunize a rabbit

Concetti (14) attempted, also unsuccessfully to immunize guinea pigs with repeated subcutaneous injections of culture filtrates of *Oidium albicans* against the same fungus injected intraperitoneally

The same author injected subcutaneously into rabbits increasing doses of a saline suspension of a sediment remaining after grinding of dried *Oidium* cells in a mortar The highest dose of this suspension was 2 cc. the weight of solids contained in this volume was not given. After an unspecified length of time rabbits were challenged intravenously with 2 cc. of a viable *Oidium* culture an amount which killed controls in 2 to 5 days. All six treated rabbits survived and manifested only a slight loss of weight

These successful experiments are in agreement with own experimental results in which suspensions of lyophilized washed cell walls were used for active immunization of mice (Figure 1)

(h) *Active Immunization of Animals Already Infected with Candida*

Attempts have also been made to induce active immunity against *Candida* by vaccine treatment in animals after they had been infected with the fungus.

Concetti (14) infected two rabbits by intravenous injections of 2 cc. of a viable culture of *Oidium albicans* One day later he started treating these animals with increasing doses of a saline suspension of a sediment remaining after grinding of dried *Oidium* cells. Daily injections were given for 4 days, starting with 1/4 cc. the highest amount being 1 cc. While two control animals died on the second and third day respectively one treated rabbit died on the 7th, the other on the 8th day Lesions caused by *Oidium* were less pronounced in treated animals than in controls.

Fomina and Stepanishcheva (34) infected mice with 1.5 million living cells of *Candida albicans* Two hours later the authors started treating the animals by active immunization with a suspension of heat killed *Candida* cells which was administered intravenously and subcutaneously every 1 to 3 days for 1 1/2 months. The doses ranged from 200 000 to 8,000,000 cells. One and a half months after the experiment was started 28 % of the vaccine-treated mice survived while there were only 8 % survivors in the control group

The experiments of Concetti and Fomina and Stepanishcheva suggest that vaccine therapy of moniliasis may be feasible

(B) *Passive Immunization*

Passive immunization was attempted in rabbits and mice. del Pont (16) treated rabbits with cultures of *Endomyces albicans* which had been attenuated by heating at 55 C for 10 minutes. The minimal lethal dose of the unheated culture of this strain of the fungus was 0.2 cc. The author injected increasing amounts of the heated suspension of *Endomyces* every 6 to 7 days for an unspecified length of time, starting with 0.2 cc. until he reached 5 cc.

The serum of animals thus treated was used for intravenous injections to four rabbits. They received 1 2, 5 and 10 cc. of the immune serum, respectively Twenty-four hours later they were challenged intravenously together with one control animal which did not receive any serum, with 0.5 cc. of a virulent culture of the same fungus. The untreated control rabbit died five days later The animal which received 5 cc. of the serum died 4 days after challenge with typical symptoms of moniliasis. The rabbit which was passively immunized with cc of immune serum died 25 days after challenge, apparently of an unrelated bacterial infection. The other two rabbits remained in good health for the duration of the experiment which was 4 months.

These experiments suggest that most of the animals may have been protected against lethal challenge by pretreatment with passively transferred antibodies. The rabbit which received 5 cc. of serum and died may have become sufficiently hypersensitive by passive transfer and susceptible to challenge

Hoffmeister Dickgiesser and Göttung (48) used the serum of two of the four rabbits which were actively immunized by a combined schedule of injections of heat-killed and living cells of *Candida albicans* which was described in a previous section (pages 36—37) The authors injected 20 cc. of this immune serum intravenously to each of two rabbits together with 5 billion and 7.5 billion of living cells, respectively None of the passively immunized animals showed any signs of the disease after 30 days while one control rabbit died in 4 another in 7 days.

In these experiments the immune serum and challenge were both given simultaneously by intravenous route. It is possible that the antiserum agglutinated the living cells and reduced thus the size of the inoculum.

Hurd and Drake (53) used serum withdrawn from 2 rabbits which were actively immunized over a period of almost 9 months by repeated injections of a vaccine prepared from *Candida albicans treated by heat and formalin* (page 40) The serum of these two rabbits which had an agglutinin titer of 1 1,280 and 1 2,560, respectively was injected into other rabbits. These were infected immediately after the serum injection with an amount of living cells of *C albicans* which killed 8 of 10 controls within a week. Three of five rabbits which received 1 ml. of antiserum and *Candida* died in 5 to 6 days. The two remaining animals were killed after an unspecified length of time. Four of five rabbits which were given 3 ml. of antiserum and *Candida* also died in 5 to 6 days The surviving rabbit was killed after ten days. The three

The experiments of Mourad and Friedman (89) have been discussed in the section on immunization with living cells (pages 38—40) As was pointed out there, mortality rates of mice immunized with merthiolate killed and sonically vibrated cells were lower than of those immunized with living cells.

(g) *Active Immunization with Fractions or Products of Candida Cells*

Attempts have been made to produce active specific immunity in animals by the use of fractions of cells of *Candida* or their cellular products.

Ostrovsky (99) reported that a single subcutaneous injection of 20 cc. of a culture filtrate of *Oidium albicans* did not immunize a rabbit.

Concetti (14) attempted, also unsuccessfully to immunize guinea pigs with repeated subcutaneous injections of culture filtrates of *Oidium albicans* against the same fungus injected intraperitoneally

The same author injected subcutaneously into rabbits increasing doses of a saline suspension of a sediment remaining after grinding of dried *Oidium* cells in a mortar The highest dose of this suspension was 2 cc. the weight of solids contained in this volume was not given. After an unspecified length of time rabbits were challenged intravenously with 2 cc. of a viable *Oidium* culture, an amount which killed controls in 2 to 5 days. All six treated rabbits survived and manifested only a slight loss of weight.

These successful experiments are in agreement with own experimental results in which suspensions of lyophilized washed cell walls were used for active immunization of mice (Figure 1)

(h) *Active Immunization of Animals Already Infected with Candida*

Attempts have also been made to induce active immunity against *Candida* by vaccine treatment in animals after they had been infected with the fungus.

Concetti (14) infected two rabbits by intravenous injections of 2 cc. of a viable culture of *Oidium albicans* One day later he started treating these animals with increasing doses of a saline suspension of a sediment remaining after grinding of dried *Oidium* cells. Daily injections were given for 4 days, starting with 1/4 cc. the highest amount being 1 cc. While two control animals died on the second and third day respectively one treated rabbit died on the 7th, the other on the 8th day Lesions caused by *Oidium* were less pronounced in treated animals than in controls.

Fomina and Stepanishcheva (34) infected mice with 1.5 million living cells of *Candida albicans* Two hours later the authors started treating the animals by active immunization with a suspension of heat killed *Candida* cells which was administered intravenously and subcutaneously every 1 to 3 days for 1 1/2 months. The doses ranged from 200 000 to 8 000 000 cells. One and a half months after the experiment was started 28 % of the vaccine-treated mice survived while there were only 8 % survivors in the control group.

The experiments of Concetti and Fomina and Stepanishcheva suggest that vaccine therapy of moniliasis may be feasible.

cells of *Monilia psilosis*. After a length of time which was not given two of these mice received also 0.2 cc. and 0.4 cc., respectively of serum from a patient who had a chronic osteomyelitis due to *M. psilosis*. The two which received no serum and the mouse which was given 0.2 cc. of the serum revealed lesions due to the fungus on autopsy which was performed two weeks later. The fourth mouse which had received 0.4 cc. of the serum contained no lesions.

Fomina and Stepanishcheva (34) treated a large series of mice with anti-*Candida albicans* rabbit serum diluted 1:10 with saline in daily doses increasing from 0.1 to 1 cc. These mice were injected intravenously with 25 million living cells of *C. albicans* 2 hours before the first serum injection was given. The source of the serum were rabbits which had undergone a long-term immunization with killed or living cells of *Candida*. On the twentieth day of the experiment 20—25 % of the mice were still alive but all of them died between the 30th and 40th day. All untreated control mice died between the 5th and the 9th day of the experiment.

The limited experiments of Connor (15) and those of Fomina and Stepanishcheva (34) point to the possibility that serum therapy of previously infected animals may be feasible.

(D) Immunization in Humans

No reports could be located in the literature on the preventive use of active or passive immunization in human moniliasis. Reports on the therapeutic use of vaccines prepared from *Candida* are not numerous. Their mode of action, at least in some instances, may be interpreted as desensitization rather than active specific immunization.

(1) Active Immunization

Vaccine therapy was reported by Grossi and Balog (39) who treated twelve patients suffering from pulmonary moniliasis, with increasing doses of living yeast cells of *Monilia albicans* intracutaneously and subcutaneously. The suspension contained a loopful of culture material in 3 cc. of saline solution. Injections were started with 0.1 cc., and the dosis was gradually increased to 1 cc. The authors claimed improvement or cure of all treated patients. Some of them, however apparently also received iodides.

Emdin and Finlayson (25) treated a 4-year-old boy with an ulcerative skin lesion on the upper lip and meningitis, both due to *Candida albicans*. Local measures and systemic treatment were applied. The latter consisted of penicillin, potassium iodide, intravenous gentian violet and a *Candida* vaccine. The vaccine was prepared from heat killed cells as described by Conant et al. (13). A dilution of 1:1,000 of a standardized stock vaccine (1:1,000) was used at first, commencing with 0.1 ml. and increasing the dose by this amount every second day. A more concentrated vaccine was injected later. Vaccine and iodide therapy were continued for several months. No information is given about the highest concentration of the vaccine or the total number of injections given. When the child was discharged after about 5 months of hospitalization he

surviving animals in these 2 groups had almost or completely healed lesions. Two rabbits were treated with daily intravenous injections of antiserum until death which occurred within a week. The authors concluded that passive immunization was ineffective and possibly deleterious.

They noticed, however some morphologic peculiarities of the lesions in passively immunized animals, viz. thick walled, distorted blastospores, a tendency of the organisms to form packed masses, and necrosis in the centers of some fungal lesions. A similar morphology of the lesions was found in some nonimmunized rabbits surviving for longer periods of time, possibly indicating the building up of natural antibodies.

The authors did not give enough information about the method of immunization of rabbits which were used as the source of antiserum. Although they were unable to prolong the life of the majority of passively immunized rabbits 3 of 10 animals survived for longer periods than the controls. The failure to protect most rabbits may have been caused by an insufficient amount of antiserum or a passive transfer of hypersensitivity

Ata (1) reported that mice pretreated with anti *Candida albicans* rabbit serum and challenged with 5×10^5 cells of *C albicans* had lower mortality rates than controls pretreated with normal serum. Mortality of mice injected with 0 1 cc. of anti-tuberculous rabbit serum intraperitoneally and challenged with an intravenous injection of 3×10^7 cells of *C albicans* 30 minutes later was 40 % fifteen days after the injection while controls injected with normal rabbit serum had a mortality of 80 %

The protection experiments with anti-*Candida* serum confirm the positive results of del Pont (16) The experimental results according to which mice injected with antituberculous rabbit serum were partly protected against a challenge with *Candida* may possibly be explained by the transfer of nonspecific resistance from rabbits pretreated with the Koch bacillus. This appears possible in view of the experimental results of Benacerraf Sebestyen and Schlossman (5) who found that intravenous injections of serum from endotoxin-treated mice into normal mice transfer the enhanced ability to clear *Escherichia coli* from the blood It will be mentioned later (page 57) that *Mycobacterium tuberculosis* or its extracts may modify nonspecific host resistance in a similar manner as the endotoxins of gram-negative bacteria (20)

Hasenclever and Mitchell (40) injected 29 mice intraperitoneally with 0.5 cc. of anti *Candida albicans* rabbit serum diluted 1 5 two hours before an intravenous challenge with 10^7 cells of *C albicans* This pretreatment did not protect them from the lethal effects of the challenge. It is possible that passive transfer of hypersensitivity to mice may have taken place

The experiments of the following two authors deal with serum therapy of animals previously infected with *Candida*

Connor (15) injected intraperitoneally 4 mice with 0 2 cc. of a suspension containing an unspecified number of viable

albicans killed with formol and heat. The tested persons received 5 doses containing 500 million, 1, 2, 3 and 4 billion cells, respectively in 1 cc. The injections were given every 3 days. The purpose of the study was to examine the immunological response of humans to the vaccine. It was found that with the exception of one subject an elevation of agglutinating and complement fixing titers took place in all tested persons after the treatment. The majority of them developed local erythema and infiltration at the site of the injections. General reactions were slight, consisting occasionally of moderate hyperthermia and slight headache. The previously negative skin tests became positive only in some cases, and the elevation of gamma globulin was inconsistent.

It appears that while the number of cells injected to the patients of Groni and Balog (39) and Emdin and Finlayson (25) was too small to produce specific immunity and the effect of the therapy may be probably attributed to desensitization, the dosage in the vaccine treatment of Scuro, Perrone and Zambonl (130) may have been high enough to produce specific immunization.

(ii) *Passive Immunization*

Hiatt and Martin (45) treated an adult patient with pulmonary moniliasis by the subcutaneous administration of anti-*Candida albicans* rabbit serum, beginning with 0.1 cc. of a 1 : 10 dilution. The dose was increased by 0.1 cc. daily until an amount of 0.9 cc. was reached. This series was repeated twice. The authors claimed a total remission of all symptoms following serum therapy.

Other forms of treatment were also administered prior to the serum therapy, viz. iodides, intravenous gentian violet and general supportive measures. These may have accounted for some of the improvement. The amount of serum injected to this patient was very small in comparison with that administered in the animal experiments described on pages 43—45. It is questionable whether a sufficient amount of specific antibodies was transferred passively by this serum therapy.

was in an excellent condition. The authors state that a distinct improvement was noted after a few vaccine injections, so that immunological response may have been the deciding factor in promoting recovery

The evaluation of the therapeutic effectiveness of the vaccine is difficult since several other forms of therapy were administered. It appears that the total number of cells contained in the vaccine injections was relatively small the initial number being about 1,250 and, in case the maximum amount (1 cc.) of undiluted vaccine was given, which does not seem to be probable, the highest number of dead cells administered would have been about 12,500 000 This is only a small fraction of the number of cells from which cell wall material for specific immunization of mice in own experiments was prepared, and a small fraction of the number of cells used in the few successful immunization experiments in animals [Hoffmeister Dickgiesser and Gotting (48) Mazzetti and Fissi Marraccini (83)]. It appears, therefore, that whatever effect the vaccine therapy may have had it may be attributed to desensitization rather than active specific immunization. In view of the work of Kurotchkin and Lim (68) (page 33) such desensitization may be considered helpful

Although it is difficult to estimate the exact number of cells contained in the vaccines used by Grossi and Balog (39) the same evalution may apply to their treatments.

Thiers, Coudert, Colomb, Fayolle and Moulin (140) reported good results in treating patients suffering of moniliasis with "*clasovaccins*" according to Jausion and Boidé (56) which are prepared by hydrolysis of *Candida* with sulfuric or nitric acid which is precipitated by calcium carbonate. The authors state that all allergens are destroyed by this rather brutal method of preparation but that the protective properties of *Candida* are preserved Unlike with their previous method of vaccine therapy in which they used cultures of *Candida* to which chloroform and formalin were added they did not experience any cutaneous or general allergic reactions, including abdominal pains and diarrhea. The authors maintain that there is a dissociation between the allergenic and protective properites of *Candida* Details of therapeutic results are not given.

It is possible that the claimed effectiveness of the "*clasovaccins*" of Jausion against dermatomycoses caused by *Trichophyton tonsurans T mentagrophytes T violaceum Epidermophyton floccosum* against sporotrichosis and in cases of secondary eczematoid allergic reactions due to a primary fungal focus (57) as well as the favorable results of a similarly prepared vaccine against moniliasis (140) may indeed be based on the absence of sensitizing materials and of endotoxin like substances which may enhance the growth of fungi and on the presence of cell wall material containing the specific protective antigen

Although Scuro Perrone and Zamboni (130) used normal subjects for their vaccination the data about side effects in the vaccinated subjects are useful. The authors injected 18 subjects subcutaneously with a suspension of increasing numbers of cells of *Candida*

albicans killed with formol and heat. The tested persons received 5 doses containing 500 million, 1, 2, 3 and 4 billion cells, respectively in 1 cc. The injections were given every 3 days. The purpose of the study was to examine the immunological response of humans to the vaccine. It was found that with the exception of one subject an elevation of agglutinating and complement fixing titers took place in all tested persons after the treatment. The majority of them developed local erythema and infiltration at the site of the injections. General reactions were slight, consisting occasionally of moderate hyperthermia and slight headache. The previously negative skin tests became positive only in some cases, and the elevation of gamma globulin was inconsistent.

It appears that while the number of cells injected to the patients of Gross and Balog (39) and Erndin and Finlayson (25) was too small to produce specific immunity and the effect of the therapy may be probably attributed to desensitization, the dosage in the vaccine treatment of Scuro, Perrone and Zambonl (130) may have been high enough to produce specific immunization.

(ii) *Passive Immunization*

Hiatt and Martin (45) treated an adult patient with pulmonary moniliasis by the subcutaneous administration of anti-*Candida albicans* rabbit serum, beginning with 0.1 cc. of a 1 : 10 dilution. The dose was increased by 0.1 cc. daily until an amount of 0.9 cc. was reached. This series was repeated twice. The authors claimed a total remission of all symptoms following serum therapy.

Other forms of treatment were also administered prior to the serum therapy, viz. iodides, intravenous gentian violet and general supportive measures. These may have accounted for some of the improvement. The amount of serum injected to this patient was very small in comparison with that administered in the animal experiments described on pages 43—45. It is questionable whether a sufficient amount of specific antibodies was transferred passively by this serum therapy.

(4) DISCUSSION

(a) *Differences between Natural and Experimental Systemic Moniliasis*

Laboratory methods generally used to infect animals with *Candida* do not reflect accurately the processes taking place in human generalized moniliasis. An experimental model in which systemic moniliasis in animals would be produced in a similar manner as in man namely by generalization through invasion of the blood stream from the gastro-intestinal tract, has not been designed. Animals fed with *Candida* by mouth cannot be infected regularly Most investigators have used an experimental model in which animals have been infected with *Candida* either intravenously or intraperitoneally In this way generalization takes place much more acutely than in natural human infection. It can be expected that even good specific immunity might not prevent the death of some animals in which acute generalized moniliasis was produced by an injection of a massive inoculum directly into the blood stream.

(b) *Factors Influencing the Outcome of Specific Immunization*

Of the many reviewed immunization experiments only a few those containing adequate data, may be evaluated

(1) The *endotoxin-like substance* of *Candida* contained in the vaccine injections is a factor which may have either a favorable or an adverse effect on the result of immunization. The resistance-influencing functions of this endotoxin like substance contained in the soluble extract of *Candida* have not been heretofore systematically explored. They are described in the experimental part of this paper (Figures 2, 3 4 5 6 and 9) Own experiments reveal that the effects of the endotoxin-like substance of *Candida* and of the endotoxins of gram negative bacteria on nonspecific resistance are similar The role of the latter in nonspecific resistance will be briefly reviewed on pages 57—58

(a) When animals are immunized with living cells of *Candida* the endotoxin-like substance from these viable cells may enhance the growth of the fungus contained in the challenge dose or in each subsequent vaccine dose containing living organisms provided the level of this endotoxin like substance is high in the tissues at the time of such an injection (pages *28—31*) In such instances this endotoxin like substance may have an adverse effect on the results of immunization.

This may be true of the experiments in which Cavallero (*10*) immunized rabbits with living cells (pages *36, 50* and 51) The lack of protection in the experiments of Ninni and Fittipaldi (96) in which all guinea pigs receiving two large intraperitoneal injections of *Candida* given *10* days apart died while

2/3 of the animals succumbed when the interval was 30 days, may be based on this mechanism (pages 28—29 and 37—38) Higher mortalities after two large intraperitoneal injections of viable cells of *Candida* given 23 days apart as compared to mortalities after one such injection in the experiments of Fischer and Horbach (31) may have been caused, besides sensitization, by this infection-enhancing property of the endotoxin-like substance (pages 38 and 50—51) A relatively lower degree of resistance among mice immunized with living cells, as compared to those immunized with nonviable vaccines, in the experiments of Mourad and Friedman (89) may have been due to the presence of the endotoxin-like substance enhancing the growth of organisms used for immunization and challenge (pages 38—40 49—50 51 52)

(b) In some experiments the endotoxin-like substance may have played a favorable role. When vaccine injections are properly timed, the substance may enhance nonspecific resistance and potentiate the specifically immunizing role of the cell walls. An own experiment has shown that it is not destroyed by heat (Figure 4) and it is, therefore present in heat-killed vaccines.

In the experiments of Hoffmeister Dickgiesser and Götting (48) (pages 36—37 and 51) the first three injections contained dead cells only The enhancing role of the endotoxin-like substance was thus eliminated. The growth of organisms administered in the fourth injection which contained living cells may have been depressed by the endotoxin-like substance in the third injection which was given 7 days earlier This is probable especially since it was administered intravenously by a route by which the resistance-influencing effects of the endotoxin-like substance are relatively pronounced. Specific antibodies may have been instrumental in depressing the growth of organisms in the last two injections. It appears, therefore, that the success of this experiment may have been due to a combination of effects of specific and nonspecific immunity

Since there was an interval of 12 days between the last vaccine injection and the challenge dose in the experiments of Mazzetti and Fini Marraccini (83) (pages 41 and 51) who used dead cells in guinea pigs it is possible that the last vaccine injection may have enhanced resistance to the challenge. The protection achieved in these experiments may thus have also been due to a combination of mechanisms of specific and nonspecific immunity

Two preparations of dead cells (sonically fibrated and merthiolate-killed) were more effective for immunization of mice than viable cells of *Candida* in the experiments of Mourad and Friedman (89) (pages 38—40, 51 and 52) A reason for better results of vaccination with nonviable cells may have been that the endotoxin-like substance in the dead cells could not have enhanced the growth of organisms used for immunization, since it was not constantly produced. The endotoxin-like substance may have even increased nonspecific resistance to the intravenous challenge since an injection of the

—445667 Dabw

vaccine was also given 5 days before challenge. A combination of the effects of specific and nonspecific resistance may have again been responsible for the results.

Of the two nonviable vaccines considerably better results were obtained with the sonically vibrated cells. Easier availability of the specific antigen in the cell wall may have been one reason for it (page 52). It is possible, however that some of the endotoxin-like substance may have been released by sonic vibrations and may have become more easily and more promptly available from this preparation than from intact merthiolate-killed cells. Although the injections were given subcutaneously the substance may have increased nonspecific resistance to intravenous challenge, especially since an injection was given also 5 days prior to challenge.

(2) *Allergic sensitization* or its absence is another factor capable of influencing the success of immunization against moniliasis. Henrici (44) postulated that the dissemination of lesions in fungus diseases resulted from hypersensitivity. He believed that the marked tendency to give rise to hypersensitivity was the most outstanding characteristic of fungus diseases which distinguished them from most of the other microbial diseases. This points to the relative importance of the elimination of the allergenic fractions of the organisms from antifungal, as compared to antibacterial vaccines, and to the importance of anatomical and chemical identification of the specific antigens in fungi.

It was shown experimentally that hypersensitivity may be one of the factors that may lead to generalization of *Candida* infections. Kurotchkin and Lim (68) have demonstrated that intratracheal induction of bronchopulmonary moniliasis was possible only after sensitization of rabbits by previous administration of heat killed cells. Ninni and Fittipaldi (96) have shown that, when intraperitoneal injections are properly timed in guinea pigs, immunization with living or heat killed cells may induce marked hemorrhagic lesions in the peritoneum. The experiments of Vogel and Krehl (145) demonstrated hemorrhagic lesions of the lungs following sensitization with heat killed cells. Kesten and Mott (61) were able to sensitize animals actively and passively to *Candida*. According to Kurotchkin and Lim (67) sensitization is less pronounced after treatment with vaccines prepared with formalin. It was shown in the experimental part of this paper that an anaphylactic shock may be produced with the soluble extract of *Candida* but not with the cell walls (Table 1). It may be concluded therefore, that the sensitizing substance from the soluble extract should be excluded from an effective vaccine.

(a) The lack of success of immunization due to sensitization may apply to the experiments of Cavallero (10) (pages 36, 48 and 51) who immunized rabbits with either a single intravenous injection of viable cells followed 10—15 days later by a challenge, or with 3 intravenous or subcutaneous injections of viable or heat killed cells given every 4—5 days followed by a challenge 30—45 days later. The same explanation may apply to the experiments of Fischer

and Horbach (31). These authors have shown that the number of controls dying after one large intraperitoneal injection of living *Candida* cells is much lower than that of mice dying after the second of two such injections if it follows the first one 23 days later (pages 38 and 49).

(b) At least a partial elimination of allergic sensitization, on the other hand, may have been responsible for the success in some experiments. The use of formalin-killed cells by Mazzetti and Fini Marraccini (83) (pages 41 and 49) may have decreased the ad erse role of sensitization. The treatment of fungal cells with sulfuric acid n the accine therapy of Thiers, Coudert, Colomb, Fayolle and Moulin (140) (page 46) may have destroyed the sensitizing materials. There is a possibility that sensitization may not have devel oped because of the gradual increase in dosage and change in route of administration from subcutaneous to intra enous in the experiments of Hoffmeister Dickgiesser and Götting (48) (pages 36—37 and 49).

(3) The *oute f administ atio* of the vaccines is also important.

(a) The fact that the endotoxin-like substance is much more effective in modifying infections when administered intravenously or intraperitoneally than by the subcutaneous route (62 and Figure 10) and that intraperitoneal or intravenous injections of viable organisms may cause generalized moniliasis more easily than subcutaneous ones, may be partly responsible for the lack of success in some immunization experiments.

This may apply to the intraperitoneal injections of living cells in rabbits of Cavallero (10) (pages 36 48 and 50) intraperitoneal injections of living cells in guinea pigs of Ninni and Fitipaldi (96) (pages 37—38 and 48—49) intra peritoneal injections of li ing cells in mice of Fischer and Horbach (31) (pages 38, 49 and 50— 1).

(b) All injections in successful immunization trials were given subcutaneously except for 4 of 6 injections in the experiments of Hoffmeister Dickgiesser and Götting (48) (pages 36—37 and 49) who started immunizing rabbits subcutaneously and continued by intravenous route.

(4) *Dead cells or their f actions* were used either throughout the immunization or at least during its early stage in all uccessful experiments. The only exception is one of the experiments of Mourad and Friedman (89) (pages 38—40, 49 and 52) in which slight protection was achieved by subcutaneous injections of viable cells of *Candida*. When dead cells are used a constant production and liberation of the endotoxin-like substance by the cells contained in the vaccine is not possible and its depressing action on nonspecific re sistance in thus eliminated.

(5) Another factor necessary for a favorable outcome of immunization is a *sufficient quantity and relative ease of availability of the specific protective antigen.*

The limited experiments of Concetti (14) (page 42) and own findings (Figure 1) demonstrate that cell walls alone may protect against challenge and that other parts of the cell are ap-

vaccine was also given 5 days before challenge. A combination of the effects of specific and nonspecific resistance may have again been responsible for the results.

Of the two nonviable vaccines considerably better results were obtained with the sonically vibrated cells. Easier availability of the specific antigen in the cell wall may have been one reason for it (page 52). It is possible, however that some of the endotoxin-like substance may have been released by sonic vibrations and may have become more easily and more promptly available from this preparation than from intact merthiolate-killed cells. Although the injections were given subcutaneously the substance may have increased nonspecific resistance to intravenous challenge, especially since an injection was given also 5 days prior to challenge.

(2) *Allergic sensitization* or its absence is another factor capable of influencing the success of immunization against moniliasis. Henrici (44) postulated that the dissemination of lesions in fungus diseases resulted from hypersensitivity. He believed that the marked tendency to give rise to hypersensitivity was the most outstanding characteristic of fungus diseases which distinguished them from most of the other microbial diseases. This points to the relative importance of the elimination of the allergenic fractions of the organisms from antifungal, as compared to antibacterial vaccines, and to the importance of anatomical and chemical identification of the specific antigens in fungi.

It was shown experimentally that hypersensitivity may be one of the factors that may lead to generalization of *Candida* infections. Kurotchkin and Lim (68) have demonstrated that intratracheal induction of bronchopulmonary moniliasis was possible only after sensitization of rabbits by previous administration of heat killed cells. Ninni and Fittipaldi (96) have shown that, when intraperitoneal injections are properly timed in guinea pigs, immunization with living or heat killed cells may induce marked hemorrhagic lesions in the peritoneum. The experiments of Vogel and Krehl (145) demonstrated hemorrhagic lesions of the lungs following sensitization with heat killed cells. Kesten and Mott (61) were able to sensitize animals actively and passively to *Candida*. According to Kurotchkin and Lim (67) sensitization is less pronounced after treatment with vaccines prepared with formalin. It was shown in the experimental part of this paper that an anaphylactic shock may be produced with the soluble extract of *Candida* but not with the cell walls (Table 1). It may be concluded therefore, that the sensitizing substance from the soluble extract should be excluded from an effective vaccine.

(a) The lack of success of immunization due to sensitization may apply to the experiments of Cavallero (10) (pages 36, 48 and 51) who immunized rabbits with either a single intravenous injection of viable cells followed 10—15 days later by a challenge, or with 3 intravenous or subcutaneous injections of viable or heat killed cells given every 4—5 days, followed by a challenge 30—45 days later. The same explanation may apply to the experiments of Fischer

and Horbach (31). These authors have shown that the number of controls dying after one large intraperitoneal injection of living *Candida* cells is much lower than that of mice dying after the second of two such injections if it follows the first one 23 days later (pages 38 and 49).

(b) At least a partial elimination of allergic sensitization, on the other hand, may have been responsible for the success in some experiments. The use of formalin-killed cells by Mazzetti and Fiori Marraccini (83) (pages 41 and 49) may have decreased the adverse role of sensitization. The treatment of fungal cells with sulfuric acid in the vaccine therapy of Thiers, Coudert, Colomb, Fayolle and Moulin (140) (page 46) may have destroyed the sensitizing materials. There is a possibility that sensitization may not have developed because of the gradual increase in dosage and change in route of administration from subcutaneous to intravenous in the experiments of Hoffmeister, Dickgiesser and Götting (48) (pages 36—37 and 49).

(3) The *route of administration* of the vaccines is also important.

(a) The fact that the endotoxin-like substance is much more effective in modifying infections when administered intravenously or intraperitoneally than by the subcutaneous route (62 and Figure 10) and that intraperitoneal or intravenous injections of viable organisms may cause generalized moniliasis more easily than subcutaneous ones, may be partly responsible for the lack of success in some immunization experiments. This may apply to the intraperitoneal injections of living cells in rabbits of Caballero (10) (pages 36, 48 and 50), intraperitoneal injections of living cells in guinea pigs of Ninni and Fittipaldi (96) (pages 37—38 and 48—49), intraperitoneal injections of living cells in mice of Fischer and Horbach (31) (pages 38, 49 and 50—51).

(b) All injections in successful immunization trials were given subcutaneously, except for 4 of 6 injections in the experiments of Hoffmeister, Dickgiesser and Götting (48) (pages 36—37 and 49) who started immunizing rabbits subcutaneously and continued by intravenous route.

(4) *Dead cells or their fractions* were used either throughout the immunization or at least during its early stage in all successful experiments. The only exception is one of the experiments of Mourad and Friedman (89) (pages 38—40, 49 and 52) in which slight protection was achieved by subcutaneous injections of viable cells of *Candida*. When dead cells are used a constant production and liberation of the endotoxin-like substance by the cells contained in the vaccine is not possible, and its depressing action on nonspecific resistance is thus eliminated.

(5) Another factor necessary for a favorable outcome of immunization is a *sufficient quantity and relative ease of availability of the specific protective antigen*.

The limited experiments of Concetti (14) (page 42) and own findings (Figure 1) demonstrate that cell walls alone may protect against challenge and that other parts of the cell are ap-

parently not needed for specific protection.

Own experiments have demonstrated that only a relatively high weight of cell wall material induces specific immunity. It was shown that while 0.1 mg and 1.0 mg. of cell walls given 3 times weekly for 3 weeks was ineffective, 5 mg protected a significant percentage of mice. One mg of cell walls was prepared from approximately 250 000 000 cells.

It should be noted that there was a rather marked difference between the results of immunization with merthiolate-killed and sonically vibrated cells in the experiments of Mourad and Friedman (89) (pages 38—40, 49—50 and 51) although the number of cells in both vaccines was the same and although no fraction of the cells was discarded in either vaccine. Several factors may have been responsible for the difference. One possibility is that sonic vibrations may have caused easier availability and therefore a more pronounced effect of the protective cell wall antigen. If this assumption is correct the antigen may possibly be expected to be located in an inside layer of the cell wall which may have been exposed to the tissues of the host as a result of the disintegration of the cells. Another possibility: an easier availability of the endotoxin like substance and its effect on increased host resistance was discussed on pages 49—50.

Although details of clinical results in the supposedly successful vaccine treatments of Thiers, Coudert, Colomb, Fayolle and Moulin (140) (page 46) are not given it is possible that the treatment of fungal cells with sulfuric acid by the method of Jausion and Bordé (55) may have not only destroyed the sensitizing materials, as the authors claim, but also the endotoxin-like substance. It may have, moreover dissolved the outer layer of the cell wall. This appears possible in view of the work of Mundkur (90) who has shown that boiling of yeast in dilute acid removed the outer layer of mannan with the associated protein. If the treatment of *Candida* with acid done by the French authors caused the same chemical change it may be expected that the removal of the outer mannan-protein or *glucomannan-protein layer would leave* the inner glucan protein layer as the active antigen in their vaccine. It was mentioned above that the same tentative conclusion about the location of the protective antigen may be reached by an evaluation of the experiment of Mourad and Friedman (89).

Vaccine therapy of animals already infected with *Candida* appears possible on the basis of the experiments of Conceti (14) (page 42) and Fomina and Stepanishcheva (34) (page 42).

It has been shown already in 1904 by del Pont (16) (page 43) that *passive immunization* of rabbits with rabbit immune serum was possible. This finding was confirmed by Hoffmeister-Dickgiesser and Götting (48) (page 43). The size of the inoculum in their experiments may have been reduced, however by a simultaneous injection of immune serum and fungal challenge. Similar experiments of Hurd and Drake (53) (pages 43—44) were essentially unsuccessful.

Both Ata (1) (page 44) and Hasenclever and Mitchell (40) (page 44) immunized mice passively with rabbit immune serum. The former author was ble to protect some animals while the experiments of the latter authors were unsuccessful.

The lack of success in some attempts to immunize passively may be due to the fact that besides protective antibodies hypersensitivity to Candida may have been passively transferred to immunized animals. This explanation may also apply to the report of Elinov and Bystrova (24) although the details of their experiments are not given. These authors stated that some mice infected with *Candida* and given anti-*Candida* serum died more rapidly than controls injected with normal serum. It is interesting in this connection that although the serum of rabbits used by Hurd and Drake (53) (pages 43—44) for passive immunization had high agglutinin titers it did not protect against challenge. As mentioned before (page 33) Kesten and Mott (61) were able to transfer sensitivity to *Monilia pilosus* passively in guinea pigs. Since Kurotchkin and Lim (68) (page 33) ha shown that sensitization predisposes to generalization of monilial infection it may be that spread of *Candida* infection in these experiments resulted f om sensitization and was not prevented by the transfer of protective antibodies or by high agglutinin titers

The experiments of Connor (15) (pages 44—45) who used human serum in mice and those of Fomina and Stepanishcheva (34) (page 45) using abbit serum in mice point to the possibility that serum therapy of animals previously infected with *Candida* may be possible.

(c) *Characteristics of the Sol ble Extract of Candida albicans*

Since own experiments have shown that the production of anaphylaxis and the depression or enhancement of nonspecif ic resistance is possible with the soluble extract of *Candida* but not with the cell wall preparation it appears that the sensitizing antigen and the endotoxin-like substance are located in the soluble cell extract. This fraction originates in the cytoplasm and possibly also in the slimy layer or capsule covering the rough outer surface of the cell wall which was demonstrated on electron microscopy by Nagata (92) Whether it contains in dition some soluble constituents of the cell wall which may have been separated from it in the course of mechanical disintegration of the cells cannot be decided without additional electron microscopic and perhaps biochemical and biophysical studies.

Because of the experimental results indicating that several apparently unrelated activities reside in the soluble extract of *Candida*, it may be fruitful to fractionate and characterize it chemically

There is a possibility that the endotoxin-like substance may be located on or close to the surface of the cell. The fact that Salvin (123) has prepared a toxic substance from *Candida albicans* by treating its yeast cells in the Mickle tissue disintegrator may support this possibility Own observations revealed that only a negligible percentage of cells

parently not needed for specific protection.

Own experiments have demonstrated that only a relatively high weight of cell wall material induces specific immunity It was shown that while 0 1 mg and 1 0 mg of cell walls given 3 times weekly for 3 weeks was ineffective, 5 mg protected a significant percentage of mice. One mg of cell walls was prepared from approximately 250 000 000 cells.

It should be noted that there was a rather marked difference between the results of immunization with merthiolate-killed and sonically vibrated cells in the experiments of Mourad and Friedman (89) (pages 38—40 49—50 and 51) although the number of cels in both vaccines was the same and although no fraction of the cells was discarded in either vaccine. Several factors may have been responsible for the difference. One possibility is that sonic vibrations may have caused easier availability and therefore a more pronounced effect of the protective cell wall antigen. If this assumption is correct the antigen may possibly be expected to be located in an inside layer of the cell wall which may have been exposed to the tissues of the host as a result of the disintegration of the cells. Another possibility an easier availability of the endotoxin like substance and its effect on *increased host resistance* was discussed on pages 49—50

Although details of clinical results in the supposedly successful vaccine treatments of Thiers, Coudert, Colomb Fayolle and Moulin (140) (page 46) are not given it is possible that the treatment of fungal cells with sulfuric acid by the method of Jausion and Boidé (55) may have not only destroyed the sensitizing materials, as the authors claim, but also the endotoxin-like substance. It may have, moreover dissolved the outer layer of the cell wall. This appears possible in view of the work of Mundkur (90) who has shown that boiling of yeast in dilute acid removed the outer layer of mannan with the associated protein. If the treatment of *Candida* with acid done by the French authors caused the same chemical change it may be expected that the removal of the outer mannan protein or glucomannan-protein layer would leave the inner glucan-protein layer as the active antigen in their vaccine. It was mentioned above that the same tentative conclusion about the location of the protective antigen may be reached by an evaluation of the experiment of Mourad and Friedman (89)

Vaccine therapy of animals already infected with *Candida* appears possible on the basis of the experiments of Concetti (14) (page 42) and Fomina and Stepanishcheva (34) (page 42)

It has been shown already in 1904 by del Pont (16) (page 43) that *passive immunization* of rabbits with rabbit immune serum was possible. This finding was confirmed by Hoffmeister Dickgiesser and Götting (48) (page 43) The size of the inoculum in their experiments may have been reduced however by a simultaneous injection of immune serum and fungal challenge Similar experiments of Hurd and Drake (53) (pages 43—44) were essentially unsuccessful

Both Ata (1) (page 44) and Hasenclever and Mitchell (40) (page 44) immunized mice passively with rabbit immune serum. The former author was able to protect some animals while the experiments of the latter authors were unsuccessful.

The lack of success in some attempts to immunize passively may be due to the fact that besides protective antibodies hypersensitivity to Candida may have been passively transferred to immunized animals. This explanation may also apply to the report of Elinov and Bystrova (24) although the details of their experiments are not given. These authors stated that some mice infected with *Candida* and given anti-*Candida* serum died more rapidly than controls injected with normal serum. It is interesting in this connection that although the serum of rabbits used by Hurd and Drake (55) (pages 43—44) for passive immunization had high agglutinin titers it did not protect against challenge. As mentioned before (page 53) Kesten and Mott (61) were able to transfer sensitivity to *Monilia psilosis* passively in guinea pigs. Since Kurotchkin and Lim (68) (page 33) have shown that sensitization predisposes to generalization of monilial infection it may be that spread of *Candida* infection in these experiments resulted from sensitization and was not prevented by the transfer of protective antibodies or by high agglutinin titers.

The experiments of Connor (15) (pages 44—45) who used human serum in mice and those of Fomina and Stepanishcheva (34) (page 45) using rabbit serum in mice point to the possibility that *serum therapy* of animals previously infected with *Candida* may be possible.

(c) *Characteristics of the Soluble Ext act of Candida albicans*

Since own experiments have shown that the production of anaphylaxis and the depression or enhancement of nonspecif ic resistance is possible with the soluble extract of *Candida* but not with the cell wall preparation it appears that the sensitizing antigen and the endotoxin-like substance are located in the soluble cell extract. This fraction originates in the cytoplasm and possibly also in the slimy layer or capsule covering the rough outer surface of the cell wall which was demonstrated on electron microscopy by Nagata (92). Whether it contains in addition some soluble constituents of the cell wall which may have been separated from it in the course of mechanical disintegration of the cells cannot be decided without dditional electron microscopic and perhaps biochemical and biophysical studies.

Because of the experimental results indicating that several apparently unrelated activities reside in the soluble extract of *Candid* it may be fruitful to fractionate and characterize it chemically.

There is a possibility that the endotoxin-like substance may be located on or close to the surface of the cell. The fact that Salvin (123) has prepared a toxic substance from *Candida albicans* by treating its yeast cells in the Mickle tissue disintegrator may support this possibility. Own observations revealed that only negligible percentage of cells

parently *not* needed for specific protection.

Own experiments have demonstrated that only a relatively high weight of cell wall material induces specific immunity. It was shown that while 0.1 mg and 1.0 mg of cell walls given 3 times weekly for 3 weeks was ineffective, 5 mg protected a significant percentage of mice. One mg of cell walls was prepared from approximately 250 000 000 cells.

It should be noted that there was a rather marked difference between the results of immunization with merthiolate-killed and sonically vibrated cells in the experiments of Mourad and Friedman (89) (pages 38—40, 49—50 and 51) although the number of cels in both vaccines was the same and although no fraction of the cells was discarded in either vaccine. Several factors may have been responsible for the difference. One possibility is that sonic vibrations may have caused easier availability and therefore a more pronounced effect of the protective cell wall antigen. If this assumption is correct the antigen may possibly be expected to be located in an inside layer of the cell wall which may have been exposed to the tissues of the host as a result of the disintegration of the cells. Another possibility, an easier availability of the endotoxin-like substance and its effect on increased host resistance, was discussed on pages 49—50.

Although details of clinical results in the supposedly successful vaccine treatments of Thiers, Coudert, Colomb, Fayolle and Moulin (140) (page 46) are not given it is possible that the treatment of fungal cells with sulfuric acid by the method of Jausion and Boidé (55) may have not only destroyed the sensitizing materials, as the authors claim, but also the endotoxin-like substance. It may have, moreover, dissolved the outer layer of the cell wall. This appears possible in view of the work of Mundkur (90) who has shown that boiling of yeast in dilute acid removed the outer layer of mannan with the associated protein. If the treatment of *Candida* with acid done by the French authors caused the same chemical change it may be expected that the removal of the outer mannan-protein or glucomannan protein layer would leave the inner glucan protein layer as the active antigen in their vaccine. It was mentioned above that the same tentative conclusion about the location of the protective antigen may be reached by an evaluation of the experiment of Mourad and Friedman (89).

Vaccine therapy of animals already infected with *Candida* appears possible on the basis of the experiments of Concetti (14) (page 42) and Fomina and Stepanishcheva (34) (page 42).

It has been shown already in 1904 by del Pont (16) (page 43) that *passive immunization* of rabbits with rabbit immune serum was possible. This finding was confirmed by Hoffmeister, Dickgiesser and Götting (48) (page 43). The size of the inoculum in their experiments may have been reduced, however, by a simultaneous injection of immune serum *and fungal* challenge. Similar experiments of Hurd and Drake (53) (pages 43—44) were essentially unsuccessful.

in own experiments, morphological and chemical studies including fractionation of the cell wall material and an investigation of immunologic and other properties of the various fractions would, therefore, appear to be fruitful steps toward devising a suitable vaccine which would be devoid of side effects.

Since specific protection may be demonstrated with washed isolated cell walls and not with the soluble extract, it may be concluded that the protective antigen is located in the cell wall. Washed cell walls produced, however local tissue irritation in addition to protection against challenge (page 14)

It is interesting in this connection that cell walls of some other organisms are apparently also the site of protective properties. It was shown that cell wall materials from *Brucella abortus* (Markenson, Sulitzeanu and Olitzki, 81) *Pasteurella pestis* (Keppie, Cocking and Smith, 59) *Bacterium tularense* (Shepard, Ribi and Larson, 133) *Bordetella pertussis* (Munoz, Ribi and Larson, 91) *Salmonella enteritidis* (Ribi, Milner and Perrine 109) and *Histoplasma capsulatum* (Salvin and Ribi, 126) contain substances capable of specifically immunizing against infection.

Although most of the work dealing with chemical composition and electron microscopic structure of yeasts and yeast like fungi was done with *Saccharomyces cerevisiae* some experimental results are available concerning the cell walls of the fungi of the genus *Candida*. Northcote and Horne (97) have shown that cell walls of *S. cerevisiae* are composed of protein, lipid and at least two polysaccharides, a mannan and a glucan. They have reported that the mannan is associated with a protein.

Kessler and Nickerson (60) have found that cell wall polysaccharides of *Candida albicans* occur as protein complexes. They have recovered a glucan-protein complex and two types of glucomannan-protein complexes from walls of mechanically disrupted cells of both *S. cerevisiae* and *C. albicans* and have shown a similarity of the fractions derived from the two organisms. The glucan-protein complex of *C. albicans* contained only around 1 % protein nitrogen while one of the glucomannan-protein complexes had a protein nitrogen of 3—4 %. The glucomannan-protein complexes were extracted with dilute potassium hydroxide and the glucan-protein complex constituted the insoluble residue. Bishop, Blank and Gardner (5) believe, however that the glucomannans reported were mixtures of glucan and mannan. Houwink and Kreger (51) reported that a small amount of chitin is also present in the cell walls of *Candida tropicalis*. This was confirmed by the experiments of Bishop, Blank and Gardner (5) who examined the details of the chemical structure of the cell wall polysaccharides of *C. albicans*. Kessler and Nickerson (60) found about 1 % of lipid in the cell walls of *C. albicans*.

It was mentioned in a previous chapter that the existence of at least two layers may be postulated in the cell walls of *C. albicans* in order to interpret the apparent increased antigenicity in the vaccines prepared from disrupted organisms as compared to vaccines from intact cells in the experiments performed

was disintegrated by this apparatus even after a treatment lasting several hours. It is possible, therefore, that the toxic substance in Salvin's experiments may have been released by this treatment from the cell surface. Roth and Murphy (115) released a toxic substance by ultrasonic vibration of *Candida* cells most of which remained intact. They believed that the active substance from the cell free filtrate which was lethal to mice treated with chlortetracycline originated on the surface of the cells. Isenberg Allerhand and Berkman (54) extracted with ethanol-ethyl ether or phenol a toxic substance from intact cells of *C albicans* which according to them originated on the cell surface.

The endotoxin like substance may possibly be located in the layer of slime seen on electron microscopy by Nagata (92) Negroni (93) observed a polysaccharide capsule in *C albicans* and reported that it was not easily separated by shaking of yeast cells with glass beads. It is possible that the fact that the toxicity of Salvin's extract was manifested only after the addition of an adjuvant and that the preparation of Roth and Murphy (115) was toxic only in chlortetracycline treated mice may be explained by incomplete separation of this slimy layer by mechanical means. A relatively high toxicity of the own preparation may perhaps be explained by a very thorough mechanical treatment. The question of the localization of the endotoxin-like substance may possibly be clarified on electron microscopy of cells treated by the above mentioned methods.

Since none of the toxic extracts have been chemically characterized it is impossible to decide whether or not these variously prepared substances are identical. If a similarity exists in chemical composition of endotoxins of gram-negative bacterial organisms and *Candida* this toxic substance may be a lipopolysaccharide or possibly the lipid A fraction thereof according to Westphal et al. (147)

It appears possible that at least most of the sensitizing properties are located in another fraction or fractions, probably originating in the cytoplasm and containing protein.

One of the main conclusions of the present paper which may be reached on the basis of own experimental studies and of a critical analysis of the literature is that an effective vaccine against moniliasis should not contain any portion of the soluble extract since it contains the endotoxin like substance and most or all of the fraction or fractions producing sensitization.

(d) Characteristics of Cell Walls

An ideal protective vaccine which would have to be nonviable, should, if possible contain a single antigenic component so that no local or general side effects would result from its administration. It is possible that a polysaccharidic antigen from the cell walls may fulfill these requirements. Findings and speculations which may be pertinent in the search for such an antigenic component in the cell wall will, therefore be briefly discussed.

Since possibly several unrelated immunologic and other properties may reside in the cell wall preparation used

the most likely site of the protective antigen, and that the irritating property may reside in the outer soluble layer composed of the glucomannan-protein complexes.

(e) *Role of Endotoxins in Nonspecific Resistance*

Instances of a change in host resistance to infections following the administration of various substances have been frequently described in the literature since the end of the last century (10?) In many of these findings the active components have been shown to be endotoxins of gram-negative bacteria (8, 62, 69) The infections affected by this action are those due to gram-negative bacteria (8, 62, 69) mycobacteria (19) staphylococci (19) streptococci (62) and some viruses (64 65 85 94) It was also found that substances of microbial origin other than gram-negative endotoxins, such as pertussis vaccine (19) and killed *Mycobacterium tuberculosis* or its extracts (20) pneumococcus polysaccharides (62) and zymosan (62) are capable of modifying nonspecific host resistance. Substances not derived from microorganisms, such as mucin (62) colloidal sulfur (62) and sterile milk (8) have also been found to have some resistance-influencing activity against experimental infections.

The response t administration of these substances was shown to be a biphasic change in resistance An injection of an endotoxin is followed by several hours to several days of decreased resistance after which resistance to bacterial challenge is increased for several day t several weeks. The relative and absolute duration of the two phases appears to be dependent to a certain degree on the size of the dose of the substance used to modify the infection (19 52, 69) A large dose of an endotoxin is followed by a relatively long phase of decreased resistance and a prolonged phase of increased resistance. Injections of these materials given simultaneously with or after bacterial infections markedly shortened the life of infected animals. Chronic bacterial infections may be converted into acute, at times fatal diseases under these conditions (127)

It is not in the scope of this paper to review the possible mechanisms by which endotoxins affect host reaction to infections. At present the knowledge of these mechanisms is incomplete. Among the many systems suggested to be operative in the production of the changes in host resistance the following may be mentioned the reticuloendothelial system (7 52) mobile phagocytes (12) bactericidal serum factors (52, 116) the properdin system (70) serum lysozyme (49) activation of fibrinolysis (50) inhibition of allergic reactions (84) and stimulation of antibody production (148)

The resistance-influencing substance of the soluble extract from the yeast cells of *Candida albicans* is referred to in this paper as endotoxin-like since it was shown in own unpublished experiments to exhibit, besides the resistance influencing properties reported here several other properties similar to those of the endotoxins of gram-negative bacteria. It was found e. g that the soluble extract of *Candida* caused general

by Mourad and Friedman (89). It was suggested that perhaps mechanical disintegration may have exposed an inside layer of the cell wall to the host tissues and made thus more easily available to the animals the protective antigen which may possibly be located in this layer (page 52).

Indeed, Yoshizawa (154) has shown that *C. albicans* possesses a cell wall about 150 mμ thick consisting of an outer relatively electron-dense and an inner less dense layer. This finding was confirmed by Nagata (92) who also reported that the rough outer surface of the external layer is covered with a layer of slime. Similar morphology of the cell wall of *C. albicans* was reported by Gale (37a) who found that the outer relatively electron-dense layer was about 40 mμ in thickness and that the inner electron thin area was about 200 mμ thick. The outer surface of the cell wall shown in his electron micrographs is rough.

This arrangement appears similar to that of the cell wall of *S. cerevisiae* which has been studied by several authors. Northcote and Horne (97) have shown that the cell walls of *S. cerevisiae* consist of at least two membranes one of which is made up of a glucan component. Mundkur (90) using special cytochemical methods for polysaccharides in electron microscopy of frozen dried yeast cells confirmed the existence of two layers in the cell walls of *S. cerevisiae*. He showed that the outer one which had a higher electron density was composed of mannan with an associated protein. The inner layer was shown to be comprised of glucan. Boiling in dilute acid removed mannan with the associated protein. Eddy (23) also examined the topographic localization of the two polysaccharidic components of yeast cell walls. He found that the mannan protein complex was released from the cell walls of *S. cerevisiae* by the treatment with papain. He suggested that this complex is located at the surface of the cell wall. Houwink and Kreger (51) found a network of very thin fibrils in the walls of *C. tropicalis*. Nickerson and Falcone (95) reported that the glucan component of yeast cell wall possessed the fibrillar structure.

These findings suggest that *Candida* has a cell wall consisting of two layers. The outer layer having a high electron density on electron microscopy is composed of a probably globular soluble mannan protein or glucomannan-protein complex or complexes. The inner layer of low electron density is comprised of a glucan protein complex which has probably a microfibrillar structure. It is relatively insoluble and constitutes the residue after extraction of the glucomannan-protein complexes with dilute potassium hydroxide. Extraction of the outer layer may also be possible with papain or boiling with dilute acid. The inner layer contains less protein than the outer layer.

It is quite possible that the protective antigen and the substance containing the irritating property may not reside in the same morphological or chemical component of the cell wall. In view of the mentioned facts and speculations a working hypothesis may be established that the inside layer containing the insoluble glucan-protein complex may be

Ravin, Rowley Jenkins and Fine (107) have shown by studies with labeled microorganisms that the source of the circulating endotoxin are the organisms constituting the normal gastrointestinal flora, and that this endotoxin is continuously absorbed from the intestine. It is reasonable to assume that relatively more endotoxin is suddenly liberated and absorbed in the course of antibiotic treatment as a result of massive destruction of enteric organisms which in most instances is unintentional since such therapy is usually not directed against them. Orth and Ruzicka (98) and Ruzicka (119) have shown by electron microscopy that bacterial organisms, e. g. *Salmonella typhosa* are disrupted by antibiotics and their cytoplasmic contents are leaving the bacterial cells. Mollaret (88) has demonstrated that the effect of released endotoxin during antibiotic therapy which is due to a massive destruction of microorganisms, is similar to the Herxheimer reaction. He has observed that guinea pigs infected with *Salmonella paratyphi B (S Schottmuelleri)* when treated with large doses of chloramphenicol, exhibited histologic changes in organs typical f those seen in animals injected with endotoxin. Some of the guinea pigs died as a result of the effect of endotoxin.

Fine Rutenburg and Schweinburg (28) demonstrated death of the majority of rabbits with coliform intestinal flora given a sublethal dose of thorotrast, intended to produce a blockade of the reticuloendothelial system, and administered polymyxin or neomycin either in one dose several hours after the blockade or in several doses given over 3—4 days before and in one dose after the blockade. Most of coliform-free rabbits treated the same way remained alive. Bacitracin given instead of either polymyxin or neomycin was harmless. The authors interpreted the death of polymyxin and neomycin-treated rabbits by a sudden and substantial increase in the amount of endotoxin entering the circulation as a result of the administration of the antibiotic. Death occurred because the blockaded reticuloendothelial system was unable to cope with the increased amount of endotoxin absorbed into the circulation. They believed that when there is an increase in the rate of production of the endotoxin in the intestine there is also an increased amount absorbed. No endotoxemia or death could be produced by bacitracin in rabbits with coliform flora, apparently since this flora is not sensitive to this antibiotic. Neomycin or polymyxin in coliform-free animals caused only a few deaths since the size of the pool of endotoxin in the intestine was reduced. The authors attributed the death of the few animals to the relativity of the term coliform-free

It is obvious that besides these toxic, shock-producing and lethal effects the endotoxins which had been released and bsorbed as a result of antibiotic treat ment may exhibit some of their other properties, among them the effect on the nonspecific resistance to infection. Since it was shown in experiments reported in this paper that *Candida* infections are enhanced by bacterial endotoxin injected *after* an infecting dose of *Candid* had been given (Figures 7 and 8) it ppears highly probable that the

toxicity and was lethal to mice when injected intravenously or intraperitoneally in suitable doses. Its hematologic effects in mice included the production of leukocytosis with relative lymphopenia. Alymphocytosis was produced after repeated administration. It was also capable of prolonging life of mice with transmitted L1210 leukemia and with the spontaneous leukemia in AKR mice. Generalized loss of hair was observed after repeated administration.

The experiments reported in this paper demonstrate that modification of *Candida* and *Salmonella* infections in mice by endotoxins of gram negative bacteria and by the endotoxin-like substance of *C albicans* is comparable, both qualitatively and quantitatively

It is not known whether the chemical nature and mode of action on nonspecific resistance of microbial substances other than gram negative endotoxins are similar to those of the endotoxins of gram negative bacteria. The same applies, of course, to the endotoxin-like substance of *Candida*.

(f) *Possible Role of Bacterial Resistance Influencing Substances in Pathogenesis of Moniliasis*

The effects of the endotoxins of gram-negative bacterial organisms on *Candida* have received only little attention. Gale and Sandoval (37) who injected mice with the endotoxin of *Escherichia coli* 30 minutes after they had been infected with *Candida albicans* found that their survival time was shortened. Hasenclever and Mitchell (41) have reported partial protection against a *Candida* infection in mice pretreated with *Salmonella enteritidis* or *S typhosa* endotoxin.

The fact that endotoxins of gram-negative organisms are capable of enhancing or depressing the spread of *Candida* in experimental animals, depending on the time of their administration, *may be helpful in explaining some* aspects of the pathogenicity of this yeast like organism.

(1) Among the unsolved problems which may be seen in a new light on the basis of the findings reported here which demonstrate that bacterial endotoxins affect resistance to *Candida* the most important one appears to be the question why *Candida infections are* enhanced in patients on antibiotic treatment or why persons who are asymptomatic carriers of *Candida* in the intestinal tract develop clinical moniliasis during or following antibiotic therapy

It is possible that the administration of an antibiotic, especially one with a "broad spectrum" effective also against gram-negative organisms, destroys much of the normal bacterial flora of the body *mainly of the intestinal tract*, which consists to a large extent of gram-negative endotoxin-producing organisms. *By the* sudden destruction of these organisms endotoxins may be liberated which may enhance the growth of microorganisms which are susceptible to the nonspecific resistance-influencing action of endotoxins and which are resistant to the administered antibiotic. Among them *C albicans* is one of the potentially pathogenic microorganisms commonly present in the intestinal tract and not susceptible to the action of the usually administered antibiotics.

Ravin, Rowley Jenkins and Fine (107) have shown by studies with labeled microorganisms that the source of the circulating endotoxin are the organisms constituting the normal gastrointestinal flora, and that this endotoxin is continuously absorbed from the intestine. It is reasonable to assume that relatively more endotoxin is suddenly liberated and absorbed in the course of antibiotic treatment as a result of massive destruction of enteric organisms which in most instances is unintentional since such therapy is usually not directed against them. Orth and Ruzicka (98) and Ruzicka (119) have shown by electron microscopy that bacterial organisms, e. g. *Salmonella typhosa*, are disrupted by antibiotics and their cytoplasmic contents are leaving the bacterial cells. Mollaret (88) has demonstrated that the effect of released endotoxin during antibiotic therapy which is due to massive destruction of microorganisms, is similar to the Herxheimer reaction. H has observed that guinea pigs infected with *Salmonella paratyphi B (S S bottmuelleri)* when treated with large doses of chloramphenicol, exhibited histologic changes in organs typical of those seen in animals injected with endotoxin. Some of the guinea pigs died as result of the effect of endotoxin

Fine Rutenburg and Schweinburg (28) demonstrated death of the majority of rabbits with coliform intestinal flora given sublethal dose of thorotrast intended to produce a blockade of the reticuloendothelial system, and administered polymyxin or neomycin either in one dose several hours after the blockade or in several doses given over 3—4 day before and in one dose after the blockade. Most of coliform-free rabbits treated the same way remained alive Bacitracin given instead of either polymyxin or neomycin was harmless. The authors interpreted the death of polymyxin and neomycin-treated rabbits by a sudden and substantial increase in the amount of endotoxin entering the circulation as a result of the administration of the antibiotic. Death occurred because the blockaded reticuloendothelial system was unable to cope with the increased amount of endotoxin absorbed into the circulation. They believed that when there is an increase in the rate of production of the endotoxin in the intestine there is also an increased amount absorbed. No endotoxemia or death could be produced by bacitracin in rabbits with coliform flora, apparently since this flora is not sensitive to this antibiotic. Neomycin or polymyxin in coliform-free animals caused only a few deaths since the size of the pool of endotoxin in the intestine was reduced. The uthors attributed the death of the few animals to the relativity of the term coliform-free

It is obvious that besides these toxic, shock-producing and lethal effects the endotoxins which had been released and absorbed as a result of antibiotic treatment may exhibit some of their other properties, among them the effect on the nonspecific resistance to infection. Since t was shown in experiments reported in this paper that *Candida* infections are enhanced by bacterial endotoxin injected after an infecting dose of *Candida* had been given (Figures 7 and 8) it ppears highly probable that the

sudden release of endotoxin from intestinal flora is responsible for the enhancement of *Candida* infections during antibiotic treatment.

One may further speculate whether another known property of the endotoxins, viz. their beneficial effect on survival after radiation observed by Smith, Alderman and Gillespie (136) may not take place in irradiated animals subjected to antibiotic treatment, as a result of the release and absorption of endotoxin from the intestinal flora caused by antibiotics. This assumption would explain more completely the experiments of Miller Hammond, Tompkins and Shorter (87) who were able to reduce mortality rates in mice exposed to x radiation by the administration of antibiotics, especially those effective against the members of the normal intestinal flora. Suppression of secondary infections in irradiated animals was apparently not the full explanation of their experiments.

A series of experiments with germ-free animals is planned to confirm the assumption that endotoxins released from intestinal flora by antibiotics enhance the growth of *Candida*. It is expected that antibiotics will not enhance the growth of *Candida* in animals which are otherwise free of microorganisms.

If this supposition is correct measures directed toward inactivation of endotoxins may be helpful in the prevention and treatment of moniliasis enhanced by antibiotic therapy Although the mechanisms of inactivation of endotoxins are not clearly understood some facts have been established.

Several workers have observed the presence, in mammalian blood (43 47 135) liver (141) and spleen (118) of factors inactivating endotoxin, which are presumably of enzymatic nature. In the experience of Rutenburg, Smith, Rutenburg and Fine (118) a splenic extract was much more effective than either liver extracts or serum. Although these factors have not been tested for their possible property of inactivating the modifying influence of endotoxins upon resistance to infections, other endotoxin-inactivating properties of these factors have been demonstrated. It was shown that the in vitro incubation of endotoxin with these substances results in an inactivation of lethality for rodents (47) tumor necrotization (71) induction of the Shwartzman phenomenon (71) pyrogenicity (43 153) and elevation of leucocytes (43) The protective effect of the human plasma was most marked in Cohn's fractions III and IV (47 153)

Rutenburg Schweinburg and Fine (117) have demonstrated that plasma in combination with reticuloendothelial macrophages is effective in the in vitro inactivation of endotoxin.

Furthermore, Takeda, Miura, Suzuki and Kasai (139) have shown that adenosine triphosphate administered intravenously in mice inactivated the lethal property and disturbances in phosphorus and lactic acid metabolism induced by endotoxins.

Janoff and Zweifach (55) have shown that after incubation of endotoxin with iron its tissue necrotizing properties in rabbits were decreased.

It may be rewarding to test the effectiveness of these factors for the in-

activation of the nonspecific stimulation of growth of *Candida* by gram-negative endotoxins. These methods may serve as a basis for a future clinical application. It is conceivable e. g., that an enzyme preparation, when isolated from animal tissues in a more purified form, may be used therapeutically. The mentioned findings may also raise the question whether patients with moniliasis enhanced by antibiotics may not benefit from blood or plasma transfusions because of the contents of an endotoxin-neutralizing substance in the blood.

(2) The development of moniliasis, at times fatal, has been described following immunization either against diphtheria, pertussis and tetanus (38) with BCG (106, 138) or with a small pox vaccine (4 58). The enhancement of the growth of *Candida* after immunization against diphtheria, pertussis and tetanus may have been due to the injection of the pertussis vaccine with known resistance-influencing properties (19). Tubercle bacilli have been shown to enhance certain bacterial infections in a similar way as the endotoxins of gram-negative bacilli (20). Dead tubercle bacilli are also used as adjuvants in biological experiments and they were found to be capable of enhancing *Candida* infections (125). Whether or not the virus of cowpox contains substances capable of enhancing infections in a similar way as the bacterial endotoxins is not known.

(3) It is not clear whether the mode of action as well as the methods of inactivation of resistance-influencing substances of microbial origin other than gram-negative endotoxins, such as those contained in the pertussis vaccine or tubercle bacilli, are identical with those applicable to the endotoxins of gram-negative bacteria. Since these substances may be responsible for the enhancement of *Candida* infections in the above described situations it may be worth while to test the earlier mentioned (pages 60—61) methods for the inactivation of endotoxins also against them.

(4) The variability of the intestinal flora of experimental animals (128) was thought to be a reason for differences in the susceptibility to bacterial infections (22). This susceptibility may be, at least partly related to the size of the intraintestinal pool of available endotoxins and resulting different opportunities to absorb endotoxins from the intestinal flora (28). It is also perhaps one of the factors responsible for the marked variations in the resistance to a standardized *Candida* infection from one animal to another and, usually even to a larger extent, from one colony of animals to another which has been seen in many own experiments. It is possible that the differences in susceptibility to infections which might have been thought to be genetically determined (36) in reality are due to differences in intestinal flora (21).

(g) *Possible Role of Resistance-Influencing Substance of Candida in Pathogenesis of Moniliasis*

It appears possible from the reviewed literature and from own experiments that one of the principal reasons for multiplication of *Candida* in systemic moniliasis may be the constant liberation of the endotoxin-like substance

sudden release of endotoxin from intestinal flora is responsible for the enhancement of *Candida* infections during antibiotic treatment.

One may further speculate whether another known property of the endotoxins, viz. their beneficial effect on survival after radiation, observed by Smith, Alderman and Gillespie (136) may not take place in irradiated animals subjected to antibiotic treatment, as a result of the release and absorption of endotoxin from the intestinal flora caused by antibiotics. This assumption would explain more completely the experiments of Miller Hammond, Tompkins and Shorter (87) who were able to reduce mortality rates in mice exposed to x radiation by the administration of antibiotics, especially those effective against the members of the normal intestinal flora. Suppression of secondary infections in irradiated animals was apparently not the full explanation of their experiments.

A series of experiments with germ-free animals is planned to confirm the assumption that endotoxins released from intestinal flora by antibiotics enhance the growth of *Candida*. It is expected that antibiotics will not enhance the growth of *Candida* in animals which are otherwise free of microorganisms.

If this supposition is correct measures directed toward inactivation of endotoxins may be helpful in the prevention and treatment of moniliasis enhanced by antibiotic therapy. Although the mechanisms of inactivation of endotoxins are not clearly understood, some facts have been established.

Several workers have observed the presence, in mammalian blood (45 47 135) liver (141) and spleen (118) of factors inactivating endotoxin, which are presumably of enzymatic nature. In the experience of Rutenburg, Smith, Rutenburg and Fine (118) a splenic extract was much more effective than either liver extracts or serum. Although these factors have not been tested for their possible property of inactivating the modifying influence of endotoxins upon resistance to infections, other endotoxin inactivating properties of these factors have been demonstrated. It was shown that the in vitro incubation of endotoxin with these substances results in an inactivation of lethality for rodents (47) tumor necrotization (71) induction of the Shwartzman phenomenon (71) pyrogenicity (43 153) and elevation of leucocytes (43). The protective effect of the human plasma was most marked in Cohn's fractions III and IV (47 153).

Rutenburg Schweinburg and Fine (117) have demonstrated that plasma in combination with reticuloendothelial macrophages is effective in the in vitro inactivation of endotoxin.

Furthermore, Takeda, Miura, Suzuki and Kasai (139) have shown that adenosine triphosphate administered intravenously in mice inactivated the lethal property and disturbances in phosphorus and lactic acid metabolism induced by endotoxins.

Janoff and Zweifach (55) have shown that after incubation of endotoxin with iron its tissue necrotizing properties in rabbits were decreased.

It may be rewarding to test the effectiveness of these factors for the in-

they challenged them with *C albicans*

Hedgecock (42) used nonviable fungal vaccines to enhance resistance to tuberculous in mice. All vaccines were prepared by killing the fungi with ethylene oxide. A partial protection against tuberculous infection was observed when the interval between intraperitoneal injection of a vaccine prepared from the mycelial phase of *H c psulatum* and intravenous challenge was 7 days. Very little resistance was seen when the interval was 29 days. No change in the course of the tuberculous infection was observed when the yeast phase of *H apsulatum* was used instead of the mycelial phase. Vaccines prepared from the mycelial phase of *Blastomyces dermatitidis* yeast cells of *Cryptococcus neoformans* yeast cells or mycelia of *Sporotrichum S henkn* given 13 days prior to infection with *Mycobacterium tuberculosis* also enhanced resistance of mice.

The following experiments of Salvin (124) strongly suggest the possibility of an endotoxin-like substance in *H psulatum* and its effect on nonspecific resistance. I a group of mice injected intraperitoneally with 10^{6} cells of *H capsulatum* in 5 % mucin 86 % died within 21 days. Of those dying in the first 3 weeks 73 % succumbed between the 4th and the 10th day after the injection but only a few died within 48 hours.

Another group of mice was injected intraperitoneally first with 10^{4} cells of *Hist plasma* and 2 weeks later with 10^{6} cells of *Hist plasma* in mucin intraperitoneally. Sixty-one per cent of them died during the first 3 weeks after the second injection. Of these 90 % died during the first 48 hours. When the size of the first injection was increased to 10^{6} cells a 4- to 5-day interval between the two injections was sufficient to produce a high percentage of early deaths.

In another experiment acetone-dried cells of *H capsulatum* were injected intraperitoneally instead of the first injection of living cells, and were followed by 10^{6} living cells 2 weeks later. The number of early deaths was not higher than among the controls, but a resistance to lethal challenge developed in pretreated mice.

Another group of mice was pretreated with 10^{6} cells of *Histoplasma* and challenged with acetone-dried cells in mucin 2 weeks later. Fifty three percent of them died within 48 hours after the second injection.

Salvin interpreted the early deaths as being due to hypersensitivity. No adequate explanation may be found, however on this basis for the absence of early deaths in mice pretreated with dead cells.

On the basis of these experimental results one may postulate that *Histoplasma*, similarly as *Candida* may contain an endotoxin-like substance effective in enhancing or depressing nonspecific resistance. In the first experiment the endotoxin-like substance constantly liberated by the cells administered in the first injection may have enhanced the growth of cells in the second injection. With a relatively small primary inoculation (10^{4} cells) it took apparently several days for the inoculum to multi-

contained in the soluble fraction of the fungus. This substance may keep enhancing the growth of the fungus and nullify the effect of the specific protective antigen of the cell walls. The same may be true of other systemic mycoses, such as histoplasmosis. The fact that cases of wide-spread bloodborne moniliasis as well as those with extensive gastrointestinal localization can be influenced by the present methods of treatment only with the utmost difficulty may possibly be explained, at least partly by this assumption.

Supporting this supposition is an own experiment in which an injection of the soluble extract of *Candida* given to mice 3 hours after an infection with *Candida* resulted in a shortened survival time (Figure 9). The same is true of the experiments of Ninni and Fittipaldi (96) in which guinea pigs were killed by living *Candida* cells injected repeatedly in several small doses the total of which was smaller than one single sublethal dose (page 28).

Provided the mechanisms of inactivation of the endotoxin-like substances of fungi are the same as those effective against endotoxins of gram-negative bacteria, which were mentioned in the preceding chapter, the experimental results concerning the inactivation of the latter may be applied in counteracting the effect of the former.

(h) *Applicability of Immunological Findings in Moniliasis to Other Fungus Diseases*

It is possible that immunological mechanisms similar to those shown here to be operative in moniliasis are at play in other fungus diseases.

(i) *The effect of endotoxins of gram negative bacteria on a fungus other than Candida* was demonstrated in the experiments of Louria (77) who has shown that resistance to *Cryptococcus neoformans* can be increased by a previous injection of a *Salmonella* endotoxin.

(ii) The *localization of protective properties in the cell wall* was reported by Salvin (124) for *Histoplasma capsulatum*.

(iii) That *cytoplasm may be the site of sensitizing substances in H. capsulatum* may be suspected on the basis of other experiments of Salvin (124a) who increased susceptibility to growth of *H. capsulatum* in mice injected with leukocytes transferred from mice which had been treated with viable or dead whole cells of *Histoplasma* or a cytoplasmic fraction thereof, but not from those which had been treated with cell walls. It is possible that passive transfer of hypersensitivity by leukocytes induced an increased susceptibility to *H. capsulatum*.

(iv) The following experiments suggest the possibility of the *presence of endotoxin-like substances* in fungi other than *Candida*. Hasenclever and Mitchell (40) (page 19) injected dead disintegrated spherules of *Coccidiodes immitis* 6 days before challenge with *Candida albicans* and found that the life of the pretreated mice was somewhat prolonged. In another set of experiments the same authors prolonged life of mice slightly by an injection of viable cells of *H. capsulatum*, *Cryptococcus neoformans*, *Coccidioides immitis* or *Aspergillus fumigatus* given 6 days before

they challenged them with *C albicans*.

Hedgecock (42) used nonviable fungal vaccines to enhance resistance to tuberculosis in mice. All vaccines were prepared by killing the fungi with ethylene oxide. A partial protection against tuberculous infection was observed when the interval between intraperitoneal injection of a vaccine prepared from the mycelial phase of *H capsulatum* and intravenous challenge was 7 days. Very little resistance was seen when the interval was 29 days. No change in the course of the tuberculous infection was observed when the yeast phase of *H capsulatum* was used instead of the mycelial phase. Vaccines prepared from the mycelial phase of *Blastomyces dermatitidis* yeast cells of *Cryptococcus neoformans* yeast cells or mycelia of *Sporotrichum Schenkii* given 13 days prior to infection with *Mycobacterium tuberculosis* also enhanced resistance of mice.

The following experiments of Salvin (124) strongly suggest the possibility of an endotoxin-like substance in *H capsulatum* and its effect on nonspecific resistance. In a group of mice injected intraperitoneally with 10^6 cells of *H capsulatum* in 5 % mucin 86 % died within 21 days. Of those dying in the first 3 weeks 73 % succumbed between the 4th and the 10th day after the injection but only a few died within 48 hours.

Another group of mice was injected intraperitoneally first with 10^6 cells of *Histoplasma* and 2 weeks later with 10^8 cells of *Histoplasma* in mucin intraperitoneally. Sixty-one per cent of them died during the first 3 weeks after the second injection. Of these 90 % died during the first 48 hours. When the size of the first injection was increased to 10^8 cells a 4- to 5-day interval between the two injections was sufficient to produce a high percentage of early deaths.

In another experiment acetone-dried cells of *H capsulatum* were injected intraperitoneally instead of the first injection of living cells, and were followed by 10^8 living cells 2 weeks later. The number of early deaths was not higher than among the controls, but a resistance to lethal challenge developed in pretreated mice.

Another group of mice was pretreated with 10^6 cells of *Histoplasma* and challenged with acetone-dried cells in mucin 2 weeks later. Fifty-three percent of them died within 48 hours after the second injection.

Salvin interpreted the early deaths as being due to hypersensitivity. No adequate explanation may be found, however on this basis for the absence of early deaths in mice pretreated with dead cells.

On the basis of these experimental results one may postulate that *Histoplasma*, similarly as *Candida* may contain an endotoxin-like substance effective in enhancing or depressing nonspecific resistance. In the first experiment the endotoxin-like substance constantly liberated by the cells administered in the first injection may have enhanced the growth of cells in the second injection. With a relatively small primary inoculation (10^6 cells) it took apparently several days for the inoculum to multi-

contained in the soluble fraction of the fungus. This substance may keep enhancing the growth of the fungus and nullify the effect of the specific protective antigen of the cell walls. The same may be true of other systemic mycoses, such as histoplasmosis. The fact that cases of wide-spread bloodborne moniliasis as well as those with extensive gastrointestinal localization can be influenced by the present methods of treatment only with the utmost difficulty may possibly be explained at least partly by this assumption.

Supporting this supposition is an own experiment in which an injection of the soluble extract of *Candida* given to mice 3 hours after an infection with *Candida* resulted in a shortened survival time (Figure 9) The same is true of the experiments of Ninni and Fittipaldi (96) in which guinea pigs were killed by living *Candida* cells injected repeatedly in several small doses the total of which was smaller than one single sublethal dose (page 28)

Provided the mechanisms of inactivation of the endotoxin-like substances of fungi are the same as those effective against endotoxins of gram-negative bacteria, which were mentioned in the preceding chapter the experimental results concerning the inactivation of the latter may be applied in counteracting the effect of the former

(h) *Applicability of Immunological Findings in Moniliasis to Other Fungus Diseases*

It is possible that immunological mechanisms similar to those shown here to be operative in moniliasis are at play in other fungus diseases.

(i) The *effect of endotoxins of gram negative bacteria on a fungus other than Candida* was demonstrated in the experiments of Louria (77) who has shown that resistance to *Cryptococcus neoformans* can be increased by a previous injection of a *Salmonella* endotoxin.

(ii) The *localization of protective properties in the cell wall* was reported by Salvin (124) for *Histoplasma capsulatum*

(iii) That *cytoplasm may be the site of sensitizing substances* in *H capsulatum* may be suspected on the basis of other experiments of Salvin (124a) who increased susceptibility to growth of *H capsulatum* in mice injected with leukocytes transferred from mice which had been treated with viable or dead whole cells of *Histoplasma* or a cytoplasmic fraction thereof but not from those which had been treated with cell walls. It is possible that passive transfer of hypersensitivity by leukocytes induced an increased susceptibility to *H capsulatum*.

(iv) The following experiments suggest the possibility of the *presence of endotoxin-like substances* in fungi other than *Candida*. Hasenclever and Mitchell (40) (page 19) injected dead disintegrated spherules of *Coccidiodes immitis* 6 days before challenge with *Candida albicans* and found that the life of the pretreated mice was somewhat prolonged In another set of experiments the same authors prolonged life of mice slightly by an injection of viable cells of *H capsulatum* *Cryptococcus neoformans* *Coccidioides immitis* or *Aspergillus fumigatus* given 6 days before

they challenged them with *C albicans*.

Hedgecock (42) used nonviable fungal vaccines to enhance resistance to tuberculosis in mice. All vaccines were prepared by killing the fungi with ethylene oxide. A partial protection against tuberculous infection was observed when the interval between intraperitoneal injection of a vaccine prepared from the mycelial phase of *H capsulatum* and intravenous challenge was 7 days. Very little resistance was seen when the interval was 29 days. No change in the course of the tuberculous infection was observed when the yeast phase of *H capsulatum* was used instead of the mycelial phase. Vaccines prepared from the mycelial phase of *Blastomyces dermatitidis* yeast cells of *Cryptococcus neoformans* yeast cells or mycelia of *Sporotrichum Schenkii* given 13 days prior to infection with *Mycobacterium tuberculosis* also enhanced resistance of mice.

The following experiments of Salvin (124) strongly suggest the possibility of an endotoxin-like substance in *H capsulatum* and its effect on nonspecific resistance. In a group of mice injected intraperitoneally with 10^8 cells of *H capsulatum* in 5 % mucin 86 % died within 21 days. Of those dying in the first 3 weeks 73 % succumbed between the 4th and the 10th day after the injection but only a few died within 48 hours

Another group of mice was injected intraperitoneally first with 10^8 cells of *Histoplasma* and 2 weeks later with 10^9 cells of *Histoplasma* in mucin intraperitoneally Sixty-one per cent of them died during the first 3 weeks after the second injection. Of these 90 % died during the first 48 hours. When the size of the first injection was increased to 10^9 cells a 4- to 5-day interval between the two injections was sufficient to produce a high percentage of early deaths.

In another experiment acetone-dried cells of *H capsulatum* were injected intraperitoneally instead of the first injection of living cells, and were followed by 10^9 living cells 2 weeks later The number of early deaths was not higher than among the controls, but a resistance to lethal challenge developed in pretreated mice.

Another group of mice was pretreated with 10^8 cells of *Histoplasma* and challenged with acetone-dried cells in mucin 2 weeks later Fifty-three percent of them died within 48 hours after the second injection.

Salvin interpreted the early deaths as being due to hypersensitivity No adequate explanation may be found, however on this basis for the absence of early deaths in mice pretreated with dead cells.

On the basis of these experimental results one may postulate that *Histoplasma* similarly as *Candida*, may contain an endotoxin-like substance effective in enhancing or depressing nonspecific resistance. In the first experiment the endotoxin-like substance constantly liberated by the cells administered in the first injection may have enhanced the growth of cells in the second injection. With a relatively small primary inoculation (10^8 cells) it took apparently several days for the inoculum to multi-

ply sufficiently to produce enough of the endotoxin like substance to enhance the growth of cells in the secondary injection. With a larger inoculum (10^8 cells) enough endotoxin like substance was apparently ready 4—5 days later to *enhance the growth of* the challenge dose.

When acetone-dried cells were used for the first injection there were no living cells present to liberate constantly the substance affecting nonspecific resistance. No early deaths took place therefore. An *increase in the resistance* to lethal challenge was observed, however This may have been due partly to the development of specific immunity and if one postulates the existence of an endotoxin like substance in *H capsulatum* partly due to the enhancement of nonspecific resistance by this substance contained in the dead cells. A similar *increase in resistance was produced by* the endotoxin like substance of *Candida albicans* (Figures 2 3 4 and 5)

Enhancement of the infection took place when dead cells were injected 2 weeks after the living organisms. If the situation in histoplasmosis is similar to that in moniliasis an endotoxin-like substance injected after challenge will *enhance the growth* of organisms contained in the challenge dose (Figures 7 8 and 9)

(v) *Conclusions.* Although the evidence of the presence *and localization* of the various immunologically active fractions in fungi other than *Candida* is fragmentary several facts may nevertheless be stated and some tentative suggestions and conclusions made on the basis of the foregoing review

(1) *Histoplasma capsulatum* (a) appears to possess an endotoxin-like substance, (b) its protective antigen may be located in the cell wall, and (c) its *cytoplasm may be the site* of sensitizing substances. If these findings are confirmed a far reaching similarity of these features with *Candida* would be demonstrated. The working hypothesis expressed for the localization of the protective antigen of *Candida* (pages 56—57) and the tentative criteria for the preparation of an anti-*Candida* vaccine *mentioned on page 54 may then* perhaps be applied and they may lead to the preparation of an effective vaccine against *H capsulatum*

(2) The presence of resistance-influencing endotoxin like substances is probable in *H capsulatum Coccidioides immitis Cryptococcus neoformans Aspergillus fumigatus Blastomyces dermatitidis* and *Sporotrichum Schenckii* Methods similar to those used in this paper for *Candida* may perhaps be utilized to confirm their presence in these fungi and examine their effect on nonspecific immunity

(3) An investigation of the presence and localization of the specific antigens and of sensitizing substances in these and other fungi may be fruitful steps toward devising effective protective antifungal vaccines. A definitive identification of the various immunologically *active fractions* of *Candida* on electron microscopy and a comparative study of *other fungi, with a search for* corresponding anatomical structures may besides *biochemical methods, lead to this* objective

(I) *Possible Application of the Reported Findings in Therapy and Prevention of Systemic Mycoses*

The knowledge of the function of some cell constituents of *Candida* reported here, and the possibility that similar mechanisms may be operative in other systemic mycoses, may help influence therapeutically by immunologic means, some of the specific and nonspecific mechanisms in systemic mycoses. This may result in a more successful treatment of these diseases. Some possible *therapeutic measures* will be discussed here.

(1) del Pont (16) Hoffmeister Dickgeser and Götting (48) Connor (15) Fomina and Stepanshcheva (34) and Ata (1) have shown that specific immunity to *Candida* may be passively transferred. A therapeutic effect in systemic mycoses may therefore, be expected from a *passive transfer of specific antibodies* by means of injections of serum from animals which had been immunized against the fungus, preferably by the use of cell walls or some of their chemical fractions, to eliminate the possible passive transfer of hypersensitivity which was demonstrated by Kesten and Mott (61)

(2) It has been realized that not much benefit could be expected from *vaccine therapy* in rapidly developing acute infections. Since, however most systemic fungus infections, including moniliasis, are usually decidedly chronic conditions, there would be time and probably need for longer lasting specific protection by active immunization. The experiments of Concetti (14) and Fomina and Stepanshcheva (34) demonstrate the feasibility of this approach. It would appear important, especially for this purpose, to use vaccines devoid of the endotoxin-like substance so that the growth of *Candida* already present in the human or animal organism is not enhanced. It would also be important to use vaccines from which allergenic products have been removed. Vaccine therapy with a cell wall preparation, if possible one containing a single antigenic component such as a cell wall polysaccharide, would, therefore, appear to be indicated as an additional therapeutic measure in systemic fungus infections.

(3) The use of measures directed toward *inactivation of the endotoxin-like substance* of *Candida* which apparently stimulates constantly the growth of the fungus in cases of extensive moniliasis (pages 61—62) has been discussed on pages 60—61 The use of these measures may apply to other systemic fungus infections as well. The importance of an attempt to *inactivate bacterial endotoxins* in systemic moniliasis enhanced by the use of antibiotics would appear obvious in the light of the discussion on pages 58—61

(4) In view of the finding of several authors that hypersensitivity is one of the possible mechanisms for the establishment of disseminated moniliasis *desensitization* may be indicated as a useful adjunct to therapy at least in some cases of systemic mycoses (82) It is possible that the cases of Grossi and Balog (39) and the patient of Emdin and Finlayson (25) may have improved as a result of desensitization with heat killed whole cells of *Candida*.

(5) A general use of *preventive vacci-*

nation against fungus diseases does not seem indicated because the exposure and susceptibility to the various fungus diseases appear to be limited to certain age groups, occupations and geographic areas. Epidemics of moniliasis do not seem to be common but they do occur especially in young infants institutionalized for long periods of time, where a danger of cross-infection with moniliasis, which may be at times fatal is high (14 26 103 142) Under special circumstances under which the danger of exposure susceptibility and occurence of epidemics of fungus diseases is high *immunization may prove to* be very useful and possibly lifesaving Immunization against histoplasmosis, coccidioidomycosis and other systemic mycoses of limited geographic distribution would apparently be useful, at least for certain segments of the population. It has been stated that the problem of coccidioidomycosis would be essentially solved by effective vaccination of susceptible persons (27) For reasons stated before cell wall vaccines should be used for this purpose providing the same general principles concerning the localization of the protective antigen, the existence and localization of endotoxin-like substances and of materials responsible for sensitization applying to *Candida* apply also to the causative agents of other systemic mycoses (page 64)

(j) *Possible Bearing of the Reported Findings on the Pathogenesis and Management of Bacterial Infections Arising During Antibiotic Treatment*

The tentative explanation for the enhancement of *Candida* infections during antibiotic therapy may have a wider application beyond the field of fungus diseases. It is conceivable that the growth of organisms other than *Candida (Pseudomonas Aerobacter Proteus Staphylococcus)* which are often not susceptible to the action of the commonly used antibiotics, may be stimulated by the endotoxins liberated from the intestinal flora which is destroyed by the action of antibiotics.

This may answer the question why these organisms which had only rarely been the cause of generalized fatal disease before the era of antibacterial drugs have been becoming increasingly dangerous since. The statistical data given by Finland Jones and Barnes (29) illustrate impressively the magnitude of this problem.

The development of adequate measures to counteract the effects of endotoxin, possibly on the basis of the work summarized on pages 60—61 may decrease the effects of this enhancement in numbers, pathogenicity and invasiveness of bacterial organisms which are not susceptible to antibiotics in patients subjected to antibiotic treatment.

(5) SUMMARY

(1) Own experiments ha e shown that a significant number of mice repeatedly injected subcutaneously with washed cell walls of *Cand da albicans* were protected against intravenous challenge w th viable cells of the same fungus which followed 21 days after the last injection. In a similar experimental setting no protection resulted from repeated injections of soluble extract of *C albicans* prepared by mechanical disintegration of its yeast cells.

(2) In a series of experiments mice were given one injection of either the endotoxin of *Escherichia coli* or the soluble extract of *C albicans* followed 5, 8, 14 and 30 days later by an intravenous challenge with *C albicans*. The survival time of mice challenged 5 8 or 14 days after the pretreatment was prolonged. The survival times of mice were shortened when either *E oli* endotoxin or the soluble extract of *C albicans* were injected after the *Candida* infection.

Similar results were obtained when the challenge with *Candida* was substituted by an intraperitoneal challenge with *Salmonella enteritidis*

The effect of the endotoxin or the *Candida* extract was much more pronounced on intraperitoneal than on subcutaneous injection. The activity of the Candida extract was preserved after heating

(3) Anaphylactic shock was produced in the majority of guinea pigs sensitized with the soluble extract of *Candida* but not with the cell walls.

(4) An extensive review of past experimental work on nonspecific host resistance, hypersensitivity and its relation to immunity and on specific immunity in moniliasis is given.

(A)( )(a) Data in the literature indicate that *Candida* infections may be enhanced by subsequent treatment with dead gram-negative bacterial organisms or their endotoxins, or by dead cells of *Saccha omyces erevisiae* A partial protection against a *Candida* infection may be achieved by pretreatment with endotoxins of *Salmon lla enteritidis* or *S typhosa*, with nonviable spherules of *Coccidioid s mmitis* sterile milk or Freund's adjuvant.

(A)(I)(b) It was shown that repeated injections of dead cells of *C albicans* given after infection with the same fungus enhance its growth while dead cells given several days before challenge have a protective effect.

(A)( )(c) Other experiments show that dead *Candida* or its extracts given simultaneously with or shortly before a challenge with *Salmonella typhosa* *Staphylococcus P oteus* *Pseudomonas* or *Mycobacterium tuberculosis* enhance the bacterial infections.

(A)(II)(a) Living *Proteus* *Pseudomonas* or *Sac haromyces* but not *St pho-*

coccus Staphylococcus or *Bacillus subtilis* injected simultaneously with or shortly after *Candida* caused much higher mortalities in mice than injections of single organisms. *Escherichia coli* injected simultaneously with *C albicans* caused only a slight increase in death rates a phenomenon possibly due to the presence in *E coli* of an antibiotic substance effective against *Candida*. A prolongation of life was noted in mice injected with *Histoplasma capsulatum Cryptococcus neoformans Coccidioides immitis* or *Aspergillus fumigatus* 6 days before challenge with *C albicans* Simultaneous infections with *C albicans* and *Mycobacterium tuberculosis* caused enhancement of the tuberculous infection. This may be due to the effects taking place in the previously mentioned experiments, viz. the presence of resistance-modifying fractions in the bacterial and fungal organisms, and to an in vitro active growth promoting polysaccharidic extract from *C albicans*

(A) (ii) (b) Small intraperitoneal injections of viable cells of *C albicans* repeated every 2—4 days killed more animals than a single large intraperitoneal injection containing a number of organisms larger than that administered in all small injections combined. When the interval between 2 large intraperitoneal injections of *C albicans* was 10 days more animals died than when the interval was 30 days. The time of death of animals intravenously infected with *Candida* was delayed when they were pretreated with the same organism by intraperitoneal route 6 days earlier

(B) Attempts to produce bronchopulmonary moniliasis by intratracheal inoculation of *Candida* were successful in animals sensitized with heat killed cells of *Candida* but not in non-sensitized animals. The authors of these experiments concluded that sensitization was one of the possible mechanisms for dissemination of moniliasis. It was also possible to sensitize rabbits passively to *Candida*.

(C) Most experiments with the aim to confer immunity to *Candida* have been unsuccessful.

(i) The reason for the lack of success may have been allergic sensitization or the presence of the endotoxin like substance of *Candida* in the immunizing injections and its adverse effect on nonspecific resistance. When viable organisms are used for immunization the endotoxin-like substance may enhance the growth of the fungus contained in each subsequent vaccine dose or in the challenge dose. In animals immunized by intravenous or intraperitoneal route this effect is more pronounced. The administration of the vaccines by these routes has another disadvantage, viz. that viable organisms in the vaccines may cause generalized moniliasis more easily

(ii) An analysis of the few successful immunization experiments reveals that dead cells were used in almost all of them at least initially This eliminated the constant liberation of the endotoxin-like substance. Easy availability of the specific antigen may have explained the success of vaccination experiments with cell walls and with sonically vibrated and acid treated cells. Sonic vibration may have caused easier availability of the protective cell wall antigen which

may be located in an inside layer of the cell wall. Treatment with sulfuric acid may have dissolved the outer mannan-protein layer and exposed the inner layer of glucan-protein which may contain the protective antigen. In some experiments the vaccine injections were timed in such a manner that it may be speculated that increase in nonspecific resistance produced by the endotoxin-like substance may have potentiated the effect of the specific protective antigen. In almost all successful experiments injections were given subcutaneously. This decreased the danger of generalization of the infection which may take place when living cells are used for immunization. The danger of sensitization may have been decreased in some experiments by the use of formalin-killed cells, in others by the treatment of yeast cells with sulfuric acid, in still others perhaps by desensitization achieved by a gradual increase of dosage and change of route of administration from subcutaneous to intravenous.

(iii) Vaccine therapy of animals already infected with *Candida* as well as passive immunization were reported to be successful. In some instances the lack of effect of passive immunization may have been due to passive transfer of hypersensitivity. Serum therapy of infected animals is also possible.

(5) The soluble fraction of *C. albicans* described in the experimental part apparently contains substances from the cytoplasm, possibly also from the capsule covering the outer surface of the cell wall and perhaps some soluble constituents of the cell wall. It produces sensitization and contains the endotoxin-like substance responsible for the modification of nonspecific host resistance. Protection against homologous challenge may be produced by the cell wall preparation which, however also causes a mild local tissue irritation. An ideal protective vaccine should contain a single antigenic component to eliminate side effects.

A critical analysis of experimental findings of others points to the possibility that the protective antigen of *C. albicans* may be located in the inside layer of the cell wall. Chemical and electron microscopic studies reveal that the outer layer of the cell wall of *C. albicans* consists of a glucomannan-protein complex while the inner layer is made up of a glucan-protein complex containing less protein than the outer layer.

(6) The administration of gram-negative endotoxins, pertussis vaccine, killed *Mycobacterium tuberculosis*, zymosan, mucin, colloidal sulfur, silica dust, sterile milk and other substances has been found to influence host resistance to gram-negative organisms, mycobacteria, staphylococci and some viruses. An injection of one of these substances is followed by several hours to several days of decreased resistance after which resistance is increased for several days to several weeks. Injections of these substances given simultaneously with or after bacterial infections shortened the life of infected animals. Chronic bacterial infections may thus be converted into acute ones.

(7) The substance present in the soluble extract of *C. albicans* which is apparently responsible for similar changes in nonspecific host resistance is referred

to as "endotoxin-like" since it has toxic and hematologic properties similar to those of gram-negative endotoxins.

(8) (a) Destruction, during antibiotic treatment, of large numbers of organisms of the normal gastrointestinal flora containing also gram negative endotoxin producing bacteria may cause sudden liberation of large amounts of endotoxin. This may similarly as an injection of endotoxin in own experiments, enhance the growth of microorganisms which are present in the host and are susceptible to the resistance influencing action of endotoxins but which are resistant to the inhibitory action of the administered antibiotic. Among them *Candida* is one of the common inhabitants of the gastrointestinal tract.

(b) The increased incidence of severe infections due to bacterial organisms such as *Pseudomonas Aerobacter Proteus* and *Staphylococcus* which used to cause such diseases only rarely before the introduction of antibiotics, may possibly be explained on the same basis.

(c) Cases of disseminated moniliasis following immunization with pertussis vaccine or BCG vaccination may be due to the action of the experimentally demonstrated resistance-influencing properties of these vaccines.

(d) One of the reasons for the multiplication of *Candida* in systemic moniliasis may be the constant liberation of the endotoxin like substance from large numbers of disintegrating *Candida* organisms.

(e) If these speculations are correct it may be worth while to try to counteract the spread of the infections enhanced by bacterial endotoxins and other resistance modifying substances by the use of measures directed toward the inactivation of endotoxins, e. g the use of serum factors active against endotoxins. Since the chemical characteristics of the resistance influencing substances other than bacterial endotoxins are unknown one may not speculate whether or not they may be inactivated by the same methods.

(9) On the basis of own experiments and of the data from the literature one may conclude that the following immunological measures may be helpful in the management of systemic moniliasis

(a) Therapy with a cell wall vaccine may be indicated for conferring a specific immunity To eliminate infection-enhancing, allergic and local side effects such a vaccine should preferably contain a single protective antigenic substance which may be located in the inner layer of the cell wall. It should not include any fractions present in the soluble extract which contains a sensitizing and an endotoxin like substance.

(b) Treatment with serum of animals immunized with a cell wall preparation may be more promptly effective and may be used as an adjunct to the active vaccine therapy The use of cell walls only for the immunization of animals may eliminate passive transfer of hypersensitivity to patients.

(c) Measures directed toward inactivation of the endotoxin-like substance of *Candida* as well as of bacterial endotoxins which may stimulate the growth of *Candida* may be helpful in patients with systemic moniliasis. These measures may or may not be identical, and

may include the use of endotoxin-inactivating serum factors.

(d) Desensitization may be a useful part of therapy since it was shown that hypersensitivity is one of the possible mechanisms for the generalization of moniliasis.

( ) A preventive use of vaccination with a cell wall preparation may be useful in certain age groups or in persons heavily exposed to the fungus.

(10)(a) The presence of resistance-influencing endotoxin-like substances is probable in *Histoplasma capsulat m Coccidioides immitis Cryptococcus neoformans Aspergillus fumigatus Blastomyces dermatitidis Sporotrich m Schenckii* and in members of the genus *Candida* other than *albicans*.

(b) It may be concluded that *Histoplasma capsulatum* has a close immunological similarity to *C. albicans*. It appears to possess, like *Candida*, not only an endotoxin-like substance, but also a protective antigen located in the cell wall and a sensitizing substance in its cytoplasm. An effective vaccine against this fungus may perhaps be prepared on the basis of the principles described here for *Candida* by using a cell wall antigen and eliminating any fractions present in its soluble extract which probably contains a sensitizing and a resistance-influencing endotoxin-like substance.

(c) An identification of the various immunologically active fractions of *Candida* on electron microscopy and a comparative electron microscopic study of other fungi, with a search for corresponding anatomical structures may besides biochemical methods, indicate whether the preparation of similar antifungal vaccines may be feasible using such fungi.

(d) Immunological measures similar to those suggested under (9) (a) to (e) for the management of systemic moniliasis may be useful in the fungus diseases the causative agents of which would reveal to ha e essential immunological features analogous to those of *Candida*. An effective preventive vaccine against histoplasmosis, coccidioidomycosis and other systemic mycoses of limited geographic distribution may solve many public health problems posed by these diseases.

ACKNOWLEDGEMENT

This ork was supported by Research Grant CA-05267-07 and by the General Research Support Grant from the National Institutes of Health of the United States Public Health Service.

to as "endotoxin-like" since it has toxic and hematologic properties similar to those of gram-negative endotoxins.

(8)(a) Destruction, during antibiotic treatment, of large numbers of organisms of the normal gastrointestinal flora containing also gram-negative endotoxin-producing bacteria may cause sudden liberation of large amounts of endotoxin. This may, similarly as an injection of endotoxin in own experiments, enhance the growth of microorganisms which are present in the host and are susceptible to the resistance-influencing action of endotoxins but which are resistant to the inhibitory action of the administered antibiotic. Among them *Candida* is one of the common inhabitants of the gastrointestinal tract.

(b) The increased incidence of severe infections due to bacterial organisms such as *Pseudomonas*, *Aerobacter*, *Proteus* and *Staphylococcus*, which used to cause such diseases only rarely before the introduction of antibiotics, may possibly be explained on the same basis.

(c) Cases of disseminated moniliasis following immunization with pertussis vaccine or BCG vaccination may be due to the action of the experimentally demonstrated resistance-influencing properties of these vaccines.

(d) One of the reasons for the multiplication of *Candida* in systemic moniliasis may be the constant liberation of the endotoxin-like substance from large numbers of disintegrating *Candida* organisms.

(e) If these speculations are correct it may be worth while to try to counteract the spread of the infections enhanced by bacterial endotoxins and other resistance-modifying substances by the use of measures directed toward the inactivation of endotoxins, e. g. the use of serum factors active against endotoxins. Since the chemical characteristics of the resistance-influencing substances other than bacterial endotoxins are unknown one may not speculate whether or not they may be inactivated by the same methods.

(9) On the basis of own experiments and of the data from the literature one may conclude that the following immunological measures may be helpful in the management of systemic moniliasis:

(a) Therapy with a cell wall vaccine may be indicated for conferring a specific immunity. To eliminate infection-enhancing, allergic and local side effects such a vaccine should preferably contain a single protective antigenic substance which may be located in the inner layer of the cell wall. It should not include any fractions present in the soluble extract which contains a sensitizing and an endotoxin-like substance.

(b) Treatment with serum of animals immunized with a cell wall preparation may be more promptly effective and may be used as an adjunct to the active vaccine therapy. The use of cell walls only for the immunization of animals may eliminate passive transfer of hypersensitivity to patients.

(c) Measures directed toward inactivation of the endotoxin-like substance of *Candida* as well as of bacterial endotoxins which may stimulate the growth of *Candida* may be helpful in patients with systemic moniliasis. These measures may or may not be identical, and

injection of pertussis vaccine or of bacterial endotoxin. J Exptl. Med. *104* 53—65.

20. Dubos, R. J and R. W Schaedler 1957 Effects of cellular constituents of mycobacteria on the resistance of mice to heterologous infections. I Protective effects. J Exptl. Med. *106* 703—717

21. Dubos, R. J and R. W Schaedler. 1960 The effect of the intestinal flora on the growth rate of mice, and on their susceptibility to experimental infections. J Exptl. Med. *111* 407—417

22. Dubos, R. J and R. W Schaedler 1962. The effect of diet on the fecal bacterial flora of mice and on their resistance to infection. J Exptl. Med. *115* 1161—1172.

23. Eddy A A 1958 The structure of the yeast cell wall. II Degradative studies with enzymes. Proc Roy Soc (London) B. *149* 425—440

24. Elinov N P and V V Bystrova. 1961 The cause of the possible death of rabbits on immunization with yeasts. J Microbiol. Epidemiol Immunobiol. (U S S R.) *32* 82—87

25. Emdin, W and M H Finlayson. 1954 Moniliasis of the central nervous system in child with recovery S. African Med. J *28* 868—871

26. Faber H K and E B Clark. 1927 Prevention and treatment of thrush (oidial stomatitis) Am. J Diseases Children *34* 408—417

27. Fiese M J 1958 Coccidioidomycosis. Charles C Thomas, Springfield, Illinois Pages *174*—*175*

28. Fine, J S Rutenburg, and F B. Schweinburg 1959 The role of the reticulo-endothelial system in hemorrhagic shock J Exptl. Med. *110* 547—569

29. Finland, M W F Jones, J and M. W Barnes 1959 Changes in the occurrence of serious bacterial infections since the introduction of antibacterial agents. Trans. Assoc. Am. Physicians *72* 305—322.

30. Fischer O W 1955. Die Soorkomplikation der Aureomycintherapie im Lichte tierexperimenteller Untersuchungen. Ann. Univ Saraviensis Serie Medizin *3* 105—166.

31. Fischer G. W and L. Horbach. 1958. Untersuchungen über Promunität und Infektionsimmunität bei der experimentellen Soorinfektion. Arch. Hyg u. Bakteriol. *142* 14—25

32. Fischer J 1959 Zur Beeinflussung der Serumbakterizidie Ein Beitrag zur Frage der unspezifischen Resistenz. Schweiz. Z. allgem. Pathol. u. Bakteriol. *22* 827—843

33. Fischl, R. 1919 Entwicklung und gegenwärtiger Stand unserer Kenntnisse über die Soorkrankheit. Ergeb. inn. Med. u. Kinderheilk. *16* 107—191

34. Fomina, I P and Z. G Stepanishcheva. 1957 Model' eksperimental'nogo ostrogo vistseral'nogo kandidomikoza myshei ee ispol'zovanie dlia izuche nia terapevticheskoi effektivnosti protivogribkovykh antibioticheskikh i khimioterapevticheskikh preparatov (An experimental model of an acute visceral moniliasis of mic and its use for the investigation of therapeutic effects of antibiotics and chemotherapeutic agents against yeasts.) Antibiotiki *2* 34—38.

35. Freyschuss, S K. L., S. O. Pehrson, and B Steenberg. 1955. Colliformin production and isolation. Antibiotics & Chemotherapy *5* 218—223

36. Gale, D and G Deverty 1957 Response of mice to inoculations of both *Candida albicans* and *Escherichia coli*. II. The effect of the strain of mice. J Infectious Diseases *101* 48—50.

37. Gale D and B Sandoval. 1957 Response of mice to the inoculations of both *Candida albicans* and *Escherichia coli* I The enhancement phenomenon J Bacteriol. *73* 616—6 4

(6) LITERATURE CITED

1 Ata, S 1957 Kreuzungsreaktion bei der Hämagglutination zwischen Candida Albicans und Mycobacterium Tuberculosis. Zentr Bakteriol Parasitenk. Abt. I Orig. 170 100—103

2 Banič, S 1960 Antagonistische Wirkung von Enterobacteriaceen auf Staphylokokken und Candida albicans. Zentr Bakteriol Parasitenk Abt. I Orig 180 27—29

3 Benacerraf B., M M Sebestyen, and S Schlossman 1959 A quantitative study of the kinetics of blood clearance of P[32]-labelled *Escherichia coli* and staphylococci by the reticulo-endothelial system. J Exptl. Med. 110 27—48.

4 Bensousan Chetboul L. 1955 Contribution à l étude du traitement des cas graves d infection à Candida albicans par la F ngicidine Medical thesis No 931 Paris

5 Bishop, C. T F Blank and P Gardner 1960 The cell wall polysaccharides of Candida albican glucan, mannan, nd chitin. Can J Chem 38 869—881

6 Bohme D 1958. Mechanismen der unspezifischen Inf ktresistenz. Klin Wochschr 36 837—845

7 Böhme D and C. A. Bouvier 1960 Übe den Einfluss bakterielle Frak tion n auf Gewebe, ret kuloendothe-liales System und Diff rentialblutbild der Albinomaus Beit path L Anat. u. allgem. Pathol. 122 188—198.

8 Brandis, H 1954 Über die Prom ität (Depressionsimmun tät) Ergeb Hyg Bakt riol. Immunitatsf rsch. u Exptl. Therap 28 141—202

9 Braude A. I and J Siemienski. 1961 The influence of endotoxin on resistance to infection. Bull. N Y Acad Med (2)37 448—467

10 Cavallero C. 1937 Observations sur la biologie du champignon du muguet » [Mycotorula albicans (Robin) Langeron et Talice 1932] Note IV Immunité actv acquise par Mycotorula albicans » Expériences de vaccination et de prémunition. Boll. sez. ital. soc. intern. microbiol. 9 262—267

11 Charrin and Ostrowsky 1896. L oïdium albicans, agent pathogène général. Compt. rend soc. biol 48 743—744.

12 Cohn Z. A., and S I Morse. 1960 Functional and metabolic properties of polymorphonuclear leucocytes. II. Influence of a lipopolysaccharide endotoxin. J Exptl. Med. 111 689—704

13 Conant N F D T Smith, R. D Baker J L. Callaway and D S. Martin. 1954 Manual of Clinical Mycology Ed. 2. W B. Saunders Company Philadelphia.

14 Concetti L. 1900 Biologie et pathogénie du muguet Arch. méd. enfants 3 449—479 517—541 590—605

15 Connor C. L. 1928. Monilia from osteomyelitis. J Infectious Diseases 43 108—116

16 del Pont, A M J 1904 Estudio sobre la inmunidad del muguet. Anales cir cul méd arg 27 331—340.

17 Dobias, B. 1957 Moniliasis in pediatrics. A M A J Diseases Children 94 234—251

18 Doromae, I and Y Kawamori. 1955 Moniliasis. J Antibiotics (J pan) Ser A 8 171—180

19 Dubos, R. J and R. W Schaedler 1956 Rev rsible changes in the susceptibility of mice t bacterial infections. I. Changes brought bout by

injection of pertussis vaccine or of bacterial endotoxin. J Exptl. Med. *104* 53—65

20. Dubos, R. J and R. W Schaedler 1957 Effects of cellular constituents of mycobacteria on the resistance of mice to heterologous infections I. Protective effects J Exptl Med. *106* 703—717

21 Dubos, R. J and R. W Schaedler 1960 The effect of the intestinal flora on the growth rate of mice, and on their susceptibility to experimental infections. J Exptl. Med. *111* 407—417

22. Dubos, R. J and R. W Schaedler 1962. The effect of diet on the fecal bacterial flora of mice and on their resistance to infection J Exptl Med. *115* 1161—1172

23 Eddy A A 1958. The structure of the yeast cell wall II. Degradative studies with enzymes. Proc. Roy Soc. (London) B. *149* 425—440.

24. Elinov N P and V V Bystrova 1961 The cause of the possible death of rabbits on immunization with yeasts J Microbiol. Epidemiol Immunobiol. (U.S.S.R.) *32* 82—87

25 Emdin, W and M. H. Finlayson. 1954 Moniliasis of the central nervous system in child with recovery S. African Med. J *28* 868—871

26 Faber H K., and E. B. Clark 1927 Prevention and treatment of thrush (oidial stomatitis) Am. J Diseases Children *34* 408—417

27 Fiese, M J 1958. Coccidioidomycosis Charles C Thomas, Springfield, Illinois Pages 174—175

28 Fine, J S Rutenburg, and F B. Schwemburg 1959 The role of the reticulo-endothelial system in hemorrhagic shock J Exptl Med *110* 547—569

29 Finland, M., W F Jones, J and M. W Barnes 1959 Changes in the occurrence of serious bacterial infections since the introduction of antibacterial agents. Trans. Assoc. Am. Physicians *72* 305—322.

30. Fischer G. W 1955. Die Soorkomplikation der Aureomycintherapie im Lichte tierexperimenteller Untersuchungen. Ann. Univ Saraviensis Serie Medizin 3 105—266.

31 Fischer G. W and L. Horbach. 1958. Untersuchungen über Promunität und Infektionsimmunität bei der experimentellen Soorinfektion. Arch Hyg. u. Bakteriol. *142* 14—25

32. Fischer J 1959 Zur Beeinflussung der Serumbakterizidie. Ein Beitrag zur Frage der unspezifischen Resistenz. Schweiz. Z. allgem. Pathol. u. Bakteriol. *22* 827—843

33 Flechl, R. 1918. Entwicklung und gegenwärtiger Stand unserer Kenntnisse über die Soorkrankheit. Ergeb. Inn. Med. u. Kinderheilk. *16* 107—191

34 Fomina, L P and Z. G Stepanishcheva. 1957 Model' eksperimental'nogo ostrogo visteral'nogo kandidomikoza myshei i ee ispol'zovanie dlia izucheniia terapevticheskoi effektivnosti protivodrozhzhevykh antibioticheskikh i khimioterapevticheskikh preparatov (An experimental model of an acute visceral moniliasis of mice and its use for the investigation of therapeutic effects of antibiotics and chemotherapeutic agents against yeasts) Antibiotiki 2 34—38

35. Freyschuss, S. K. L S. O Pehrson, and B Steenberg. 1955 Coliformic production and isolation Antibiotics & Chemotherapy *5* 218—223

36 Gale D and G Deresy 1957 Response of mice to inoculations of both *Candida albicans* and *Escherichia coli*. II The effect of the strain of mice. J Infectious Diseases *101* 48—50

37 Gale D and B. Sandoval 1957 Response of mice to the inoculations of both *Candida albicans* and *Escherichia coli* I. The enhancement phenomenon. J Bacteriol *73* 616—624

37a. Gale G R. 1963 Cytology of *Candida albicans* as influenced by drugs acting on the cytoplasmic membrane. J Bact riol. 86 151—157
38. Gausewitz P L., F S. Jones, and G Worley Jr 1951 Fatal generalized moniliasis Report of a case. Am. J Clin. Pathol. 21: 41—49
39 Groen, G and P Balog 1929 Clinical and experimental studies in Castella ni's pulmonary moniliasis. J Trop Med. Hyg 32 253—262
40 Hasenclever H. F., and W O Mitchell. 1962 Production in mice of tolerance to the toxic manifestations of *Candida albic ns* J Bacteriol 84 402—409
41 Hasenclever H. F and W O Mitchell. 1962. Production of tolerance to the toxicity of *Candida albicans* by non-fungal materials. J Bacteriol. 84 1325—1329
42. Hedgecock, L. W 1962. Effect of vaccines prepared from *Histoplasma capsulatum* and other yeasts on experimental tuberculosis. J Bacteriol. 82 115—123
43 Hegemann F 1957 Studien über die Natur des fieberstoffneutralisierenden Faktors im normalen menschlichen Bl t IV Mitt ilung Der Nachweis der endotoxinneutralisierenden Eigenschaft des menschlichen Blutserums durch den Tierversuch. Z. Immunitätsforsch 114 1—9
44 Henrici, A. T 1940 Characteristics of fungous diseases. J Bacteriol. 39 113—138.
45 Hiatt, J S and D S Martin. 1946 Recovery from pulmonary moniliasis following serum therapy J Am. Med. Assoc 130 205—206
46 Hill G A. 1958 Host resistance in systemic mycotic infections. Ph.D Thesis. Univ of Utah (L.C. Card No. Mic 58—7919) 98 p. Univ Microfilms. Ann Arbor Mich.
47 H M., and E. H. Kass. 1957 A plasma factor that protects against the lethal action of end toxin. J Clin. Invest. 36 900
48 H ffmeister W F Dickgiesser and H. Götting. 1951 Tierexperimentelle und serologische Untersuchungen zur Diagnostik und Therapie der Infektion mit Candida albicans. Deut. Arch. klin. Med. 198 499—508.
49 Hook, W A., and L. H. Muschel. 1959 Lysozyme and properdin levels in mice treated with zymosan endotoxin or x irradiation. Federation Proc. 18 573.
50 Hörder M H., B Kickhöfen, nd F Wendt. 1958. Aktivierung der Fibrinolyse beim Menschen durch ein bakterielles Pyrogen. Der Einfluss von Phenylbutazon und Heparin auf Fibrinolyse, Blutgerinnung und Fieberreaktion. Klin. Wochsch 36 164—166.
51 Houwink, A. L., and D R. Kreger 1953 Observations on the cell wall of yeasts. An electron microscope and x ray diffraction study Antonie van Leeuwenhoek. J Microbiol. Serol. 19 1— 4
52 Howard, J G D Rowley and A. C. Wardlaw 1958 Investigations on the mechanism of stimulation of non-specific immunity by bacterial lipopolysaccharides. Immunology 1 181—203
53 Hurd R. C and C. H Drake. 1953. *Candida albicans* infections in actively and passively immunized animals. Mycopathol et Mycol. Appl. 6 290—297
54 Isenberg H. D J Allerhand, and J I Berkman. 1963 An endotoxin-like fraction extracted from the cells of *Candida albicans* N ture (London) 197 516—51
55 Janoff A and B W Zweifach. 1960 Inactivation of bacterial exotoxins and endotoxin by iron J Exptl. Med. 112 23—34
56. Ja sion, H and D Boidé 1930. Les lasines Clasovaccins mélioré. Technique de préparation. Compt. rend. soc. biol. 104 455—456.
57 Jausion, H., and R. Sohier 1930. Les claso-vaccins. Vaccino-thérapie des dermatomycoses et d leurs séquelles allergiques. Presse méd. 38 621—625

58. Keller, W. 1936. Soorangina nach Impfung. Klin. Wochschr. *15* 683—684
59. Kepple, J. E. C. Cocking and H. Smith. 1958. A non-toxic complex from Pasteurella pestis which immunizes both guinea-pigs and mice. Lancet *1* 246—247
60. Kessler G. and W. J. Nickerson. 1959. Glucomannan-protein complexes from cell walls of yeasts. J. Biol. Chem. *234* 2281—2285.
61. Kesten, H. D. and E. Mott. 1931. Hypersensitiveness to soluble specific substances from yeast-like fungi. I. Anaphylaxis. J. Exptl. Med. *53* 803—814
62. Kiser J. S. H. Lindh, and G. C. de Mello. 1956. The effect of various substances on resistance to experimental infections. Ann. N.Y. Acad. Sci. *66* 312—328.
63. Kligman, A. M. and E. D. DeLamater. 1950. The immunology of the human mycoses. Ann. Rev. Microbiol. *4* 283—312.
64. Kradolfer F. and R. Wyler. 1958. Unspezifische Resistenzfaktoren bei Virusinfektionen. Schweiz. Z. allgem. Pathol. u. Bakteriol. *21* 83—88
65. Kradolfer F. R. Wyler and R. Meier. 1957. Abhängigkeit der Antivirenwirkung bakterieller Polysaccharide von Behandlungstermin, Behandlungsdosis und Infektionsstärke. Experientia *13* 187—189
66. Kurotchkin, T. J. and C. E. Lim. 1930. Anaphylaxis with water soluble specific substance from yeastlike fungi. Proc. Soc. Exptl. Biol. Med. *28* 223—225
67. Kurotchkin, T. J. and C. E. Lim. 1931. Effect of formaldehyde upon the sensitizing property of Monilia. Proc. Soc. Exptl. Biol. Med. 29 237—239
68. Kurotchkin, T. J. and C. E. Lim. 1933. Experimental bronchomoniliasis in sensitized rabbits. Proc. Soc. Exptl. Biol. Med. *31* 332—334
69. Landy, M. 1956. Increase in resistance following administration of bacterial lipopolysaccharides. Ann. N.Y. Acad. Sci. 66 292—303
70. Landy M., and L. Pillemer. 1956. Elevation of properdin levels in mice following administration of bacterial lipopolysaccharides. J. Exptl. Med. *103* 823—833.
71. Landy M., R. C. Skarnes, F. S. Rosen, R. J. Trapani, and M. J. Shear. 1957. Inactivation of biologically active (endotoxic) polysaccharides by fresh human serum. Proc. Soc. Exptl. Biol. Med. *96* 744—747
72. Lederer H. and R. McL. Todd. 1949. Thrush in infancy. Arch. Disease Childhood *24* 200—207
73. Levine H. B. 1962. Immunogenicity of experimental vaccines in systemic mycoses, p. 254—276. *In* G. Dalldorf (ed.) Fungi and fungous diseases. A symposium of the Section of Microbiology. The New York Academy of Medicine. Charles C Thomas, Springfield, Illinois
74. Lim, C. E. and T. J. Kurotchkin. 1932. Active bacterial anaphylaxis in relation to antibody production. Proc. Soc. Exptl. Biol. Med. *29* 1151—1153
75. Lim, C. E. and T. J. Kurotchkin. 1936. The length of incubation time in active anaphylaxis produced with cultures of Monilia. Chinese Med. J. Suppl. I Feb. 1936 256—260.
76. Lim, C. E. T. J. Kurotchkin, and C. J. W. 1930. Experimental bronchomoniliasis in rabbits. Nat. Med. J. China *16* 537—544
77. Louria, D. B. 1960. Specific and non-specific immunity in experimental cryptococcosis in mice. J. Exptl. Med. *111* 643—665
78. Mankiewicz, E. and M. Liivak. 1960. Effect of *Candida albicans* on the evolution of experimental tuberculosis. Nature (London) *187* 250—251.
79. Mankiewicz, E., E. Stackiewicz, and M. Liivak. 1959. A polysaccharide isolated from Candida albicans as growth-promoting factor for Mycobac

terium tuberculous. Can. J Microbiol. 5 261—267

80 Marcus, S and F R. Rambo. 1955 Comparative aspects of immunization of mice against systemic mycoses. Bacteriol. Proc. Soc. Am. Bacteriologists 55 92.

81 Markenson, J D Sulitzeanu and A. L. Ol tzkl. 1967 Immunogenic activity of Brucella cell wall. Brit. J Exptl. Pathol. 43 67—76

82 Martin D S 1941 The practical application of some immunologic principles to the diagnosis and treatment of certain fungus infections. J Invest. Dermatol. 4 471—481

83 Mazzetti, G and G Fiasi Marraccini. 1957 Primi dati sperimentali su una vaccinoprofilassi e una vaccinoterapia dell infezione da Candida albicans. Giorn mal. infettive e parassit. 9 323—325

84 Meier R., H J Bein, and R. Jacques. 1957 The action of bacterial poly saccharides on allergic phenomena Intern Arch. Allergy Appl. Immunol. 11 101—118.

85 Meier R., and F Kradolfer 1956 Sch tzw rkung von polysaccharidartigen Fraktionen bakterieller Herkunft bei einer experimentellen Virusinfek tion Experientia 12 13—214

86 Merkenschlager M K. Schlossmann and W Kurz. 1957 Ein mechani scher Zellhomogenisato und seine Anwendbarkeit auf biologische Probleme Biochem. Z. 329 332—340

87 Miller C. P C W Hammond, M Tompkins, and G Shorter 1952. The treatment of postirradiation infection with nt'biotics An experimental study on mice J Lab. Clin Med 39 46 —479

88. Mollaret P 1950 Ueber Nutzen und Gefahren des Chloramphenicols (Chloromycetin) be der Behandlung des Typhus und Paratyphus. Wien klin. Wochschr 62 381—384

89 Mourad, S. and L. Friedman. 1961 Active immunization of mice gainst Candid albicans. Proc Soc. Exptl. Biol. Med. 106 570—572

90 Mundkur B 1960 Electron microscopical studies of frozen-dried yeast. I. Localization of polysaccharides. Exptl. Cell Research 20 28—42

91 Munoz, J E. Ribi, and C. L. Larson. 1959 Antigens of Bo detella pertussis I Activities of cell walls and protoplasm. J Immunol. 83 496—501

92 Nagata A. 1962. Studies on the fine structure of Candid albicans (In Japanese) Yonago Igaku Zasshi (J Yonago Med. Assoc.) 13 4—24

93. Negroni, P 1936 Étude de la capsule de Mycotorula albicans (Ch. Robin, 1853) Ann. parasit l. humaine et comparée 14 511—516

94 Nemes, M M and M. R. Hilleman. 1962 Effect of Westphal lipid A on viral activities in mice and hamsters. Proc. Soc. Exptl. Biol Med. 110 500—504

95 Nickerson W J and G Falcone. 1959 Function of protein disulfid reductase in cellular division of yeasts, p. 409—424 I R. Benesch, (ed.) Sulfur n proteins. Proceedings of a symposi m held at Falmouth, Mass May 1958. Academic Press, New York.

96 Ninni, C., and C. Ettipaldi. 1934 Allergie et réactions d'hyperréceptivité et d immunité au cours des infections expérimentales par les mycètes blastosporés (Not IV) Boll. sez. ital. soc. intern. microbiol. 6 474—479

97 Northcote D H and R W Horne 1952 The chemical composition and structure of the yeast cell wall. Biochem. J 51 232—236

98 Orth, E. and O Ruzicka. 1950 Untersu hungen am Elektronenmikroskop über die Chloromycetin-Wirkung. Wien. med. Wochschr 100 570—571

99 Ostrovsky F 1896 Rech rches expérimentales sur l'infection générale prod it pa le hampignon d muguet. Parallèle pathogénique entre la malad mycot que et l'infection bacté

nenne. Medical thesis, Paris G Steinbell. Paris. 93 p.

100 Owen, C. R., M. B. Anderson, and A. T Henrici. 1937 Allergy in Monilia and yeast infections Mycopathologia (The Hague) *1* 10—25

101 Paine, T F 1952. *In vitro* experiments with *Monilia and Escherichia coli* to explain moniliasis in patients receiving antibiotics. Antibiotics & Chemotherapy 2 653—658.

102 Philipson, J 1937 Experimental studies on enhanced resistance to infection following some non-specific measures. Acta Pathol. Microbiol Scand. Suppl N 32 1—148.

103 Plenert, W 1955 Studie zum Verhalten von Candida in der Kinderklinik. Deut. Gesundheitsw *10* 1574—1580

104 Pospíšil, L., and Z. Vlašin. 1960. Zur Frage der serologischen Untersuchung bei oberflächlichen Candida-Infektionen Dermatologica *120* 223—230.

105 Prockasow J J 1962. Treatment of opportunistic fungus infections. Lab. Invest. *11* 1217—1230

106. Ramón Guerra, A. U J L Apratto C Fosatti, A Ferreira Apolo, P Pareyre, J E. Mackinnon, and R. C. Artagaveytia-Allende 1957 Hipogammaglobulinemia congénita familiar con moniliasis diseminada grave familiar y ebecegeítis atípica. Rev colombiana pediat puericult. *16* (special number) 102—115

107 Ravin, H. A D Rowley C. Jenkins, and J Fine. 1960. On absorption of bacterial endotoxin from the gastrointestinal tract of the normal and shocked animal J Exptl Med *112* 783—792

108 Reimann, H. A and T J Kurotchkin. 1931 Attempts to produce bronchomoniliasis in monkeys Am. J Trop. Med *11* 131—135

109 Ribi, E K. C Milner and T D Perrine 1959 Endotoxic and antigenic fractions from the cell wall of *Salmonella enteritidis*. Methods for separation and some biologic activities. J Immunol. *82*: 75—84

110. Rimbaud P J.-A. Rioux, and J M. Bastide. 1960. Les phénomènes immunologiques dans les candidoses les réactions sérologiques Bull. soc. franç. dermatol. syphilig. *67* 673—684

111 Roger M. 1896 Modification du sérum chez les animaux vaccinés contre l'oïdium albicans. Compt. rend. soc. biol. *48* 728—730

112. Roger M. 1896. L'infection oïdienne. Presse méd. *6* 105—109

113 Rosebury T D Gale and D F Taylor. 1954 An approach to the study of interactive phenomena among microorganisms indigenous to man. J Bacteriol. 67 135—152.

114 Rosen, F S. 1961 The endotoxins of gram-negative bacteria and host resistance New Engl J Med. *264* 919—923 980—985

115 Roth, F J J and W H. Murphy J 1957 Lethality of cell-free extract of *Candida albicans* for chlortetracycline-treated mice. Proc. Soc. Exptl. Biol Med. *94* 530—532.

116 Rowley D 1956. Rapidly induced changes in the level of non-specific immunity in laboratory animals. Brit. J Exptl. Pathol. *37* 223—234

117 Rutenburg S. H F B Schweinburg, and J Fine. 1960. In vitro detoxification of bacterial endotoxin by macrophages J Exptl. Med. *112* 801—807

118. Rutenburg, S. H., E E Smith, A. M. Rutenburg, and J Fine. 1962. Degradation of endotoxin by splenic extracts Antimicrob. Agents & Chemotherapy (Detroit) *1961* 142—147

119 Ruzicska, O 1951 Elektronenoptisch fassbare Wirkung der Antibiotika. Monatsschr Kinderheilk. *99* 22—24

120. Rzucidło, L. A. Stachów A. Nowakowska, and J Kubica. 1958. Chemical and biological properties of cell walls of Candida krusei, Trichophyton gypseum and Penicillium notatum. Bull acad. polon. sci Chemo II *6* 15—20.

ACTA MEDICA SCANDINAVICA

SUPPLEMENTUM 420

EFFECT OF DIGITALIS GLUCOSIDES ON ELECTROCARDIOGRAM AND EXERCISE TEST IN HEALTHY SUBJECTS

By

GUNVOR NORDSTRÖM-ÖHRBERG

ACCOMPAN

STOCKHOLM 1964

ACTA MEDICA SCANDINAVICA

has been published since 1919 as a continuation of Nordiskt Medicinskt Arkiv founded in 1869 by Axel Key The first volume of Acta Medica Scandinavica is therefore numbered LII (52)

The chief editors have been Axel Key 1869—1900 C. G Santesson 1901—1915 I Holmgren 1916—1957 and Birger Strandell 1958 to date.

Acta Medica Scandinavica publishes original research papers in the field of intern medicine. Priority will be given to authors from countries which are represented c the editorial board. Contributors from those countries should submit their papers to a representative of the country in question. Contributors from other countries should send their papers to the Editor at the address below

Submission of a manuscript for publication will be held to imply that the pape has not been published elsewhere and that, if accepted, it will not be republished ı any other journal in the same or similar form, without the Editor's written consent.

Papers will be published in English French or German at the authors choice, followed by a summary in English.

The manuscripts shall be in final form as submitted. They shall be typewritten with double spacing and broad left hand margin.

If a paper exceeds 16 printed pages, the cost for the excess pages shall be borne by the author No paper shall exceed 24 printed pages.

Subscription

The annual subscription to the journal, covering two volumes, each of 6 numbe is 140 Sw crowns or U S $ 27 25 *including postage* in the Scandinavian countri and in Holland 120 Sw crowns.

Address for subscriptions and all communications

ACTA MEDICA SCANDINAVICA

P O Box 2052 Stockholm 2

Requests for duplicate copies of numbers that have gone astray can only be considered if made within a fortnight of the receipt of the following number

From the Department of Clinical Physiology
and
the Department of Medicine
of Karolinska Institutet at Serafimerlasarettet, Stockholm, Sweden

EFFECT OF DIGITALIS GLUCOSIDES ON ELECTROCARDIOGRAM AND EXERCISE TEST IN HEALTHY SUBJECTS

BY
GUNVOR NORDSTRÖM-ÖHRBERG

STOCKHOLM 1964

Boktryckeriaktiebolaget Thule
Stockholm 1964

CONTENTS

Boktryckeriaktiebolaget Thule
Stockholm 1964

Ever since Withering published "*An Account of the Foxglove and some of its Medical Uses*" in 1785 the haemodynamic effect of the digitalis glucosides both on healthy persons and on patients with cardiac insufficiency has been the subject of extensive study. Withering's lucid thought, his brilliant style and the rich variety of his observations have however hardly been surpassed in any clinical account during the subsequent centuries.

The conception of the mode of action of the digitalis glucosides has changed considerably in the course of the years, as also whether their primary point of attack is the myocardium, the peripheral blood vessels or the kidney. Whereas there always seems to have been a consensus of opinion concerning the favourable effect of digitalis on the failing heart, the opinions on its effect on the healthy heart have greatly varied. Early reference was made to the paradoxical effect of the cardioactive glucosides on the normal circulation.

The measure of this harmful effect seems to be a lowering of the cardiac output (Stewart & Cohn 1932, MacMichael & Sharpey-Schafer 1944, Bing et al. 1950, Harvey et al. 1951, Williams et al. 1958). By increasing the diastolic tone of the heart, digitalis would appear to prevent its normal filling during diastole, so resulting in a smaller cardiac output (Stewart & Cohn 1932). Another view which was first advanced in conjunction with animal experiments by Dock & Tainter* in 1930 and in 1944 after experiments on man by MacMichael & Sharpey-Schafer was that the primary cause of the reduced cardiac output was a lowering of the central venous pressure, that in fact the primary effect of the cardioactive glucosides was to be sought on the venous side, an opinion which Lagerlöf & Werkö (1949) proved to be untenable.

The mechanical efficiency of the heart was also thought to decrease in healthy subjects (Erickson & Fahr 1945, Bing et al. 1950). "Digitalis acted as a cardiac poison" (Erickson & Fahr).

This negative attitude to the effect of the digitalis glucosides on the healthy or non-insufficient heart soon changed. As early as 1924 Luten had presumed that digitalis increased the muscular efficiency of the heart muscle. Later in experiments on the isolated mammalian heart, it was shown that digitalis increases the force of contraction of the heart (Wiggers & Stimson 1927, Cattell & Gold 1938). That this applies also to the intact heart has since been proved by several authors (Walton et al. 1950, Cotten & Stopp 1958, Braunwald et al. 1961). According to this view digitalis stimulates the force of systolic contraction of the healthy heart, with consequent increase of blood pressure at an unchanged peripheral resistance. The increase in force of contraction need in no way be reflected in changes of cardiac output. In actual fact several investigators have shown that digitalis exerts no haemodynamic action on the healthy or non-insufficient heart (Liljestrand & Nylin 1941, Lagerlöf & Werkö 1949, Werkö et al. 1958, Dresdale et al. 1959, Selzer et al. 1959). In acute-phase experiments with lanatoside C, Rodman et al. (1961) showed a transient reduction of the resting

* Their paper contains an excellent bibliography of the older literature on the subject.

intravenously into 20 young healthy persons. The exercise was light judging from the heart rate. It was done on a treadmill and one chest lead was recorded. Yu et al., too, found ST T changes which at times were extremely pronounced.

These three studies, which appear to be the only ones relating to the effect of digitalis on the exercise ECG have comprised few observations and a relatively unstandardized test in which the exercise was quite light. In the only study dealing with the ECG reaction also during exercise (Yu et al.) the system of ECG recording was unsatisfactory

In view of the very incomplete study of the effect of the digitalis glucosides on the circulatory response and on the ECG reaction during muscular exercise, these problems have been taken as the subject of the present study For this purpose the form of exercise has been used which was introduced by Sjöstrand in 1947 and has since been employed in several other Swedish investigations (Wahlund 1948, Holmgren 1956, Bengtsson 1956 b, Linroth 1957 Sandberg 1957 and 1961 and others)

cardiac output in young healthy subjects this effect lasted about one hour The same transient effect was evident from the study made by Harvey et al. (1951) who found the cardiac output to be reduced within one hour but thereafter restored.

The effect of digitalis during exercise has been studied to a comparatively little extent. In 1952 Yu et al. showed that light exercise on a treadmill could be performed at a lower heart rate after digitalization, while in 1958 Nordstrom-Öhrberg reported that acute-phase administration of cardioactive glucosides did not affect the working capacity or certain circulatory parameters in healthy subjects. Determination of cardiac output during exercise was done by Williams et al. (1958) in acute phase experiments. They found a lowered cardiac output. The experiments were made within one hour of digitalization and accorded in time with the transient effect on the cardiac output observed by Rodman et al (1961) who however in experiments lasting two days, found no influence on the cardiac output either at rest or during exercise. Schröder et al (1962) arrived at the same results in experiments with prolonged digitalization of healthy persons.

Digitalis glucosides have an effect also on the peripheral vascular bed It was found at an early stage that digitalis provoked a rise of pressure in the systemic circulation (Dock 1930 Katz et al. 1938) Later Ross et al. (1960) made an extensive study of the effects of digitalis on the peripheral circulation, which revealed that the earlier observed rise of blood pressure was caused partly by a raised peripheral resistance owing to a direct effect on the smooth musculature in arterioli. This increase of the peripheral resistance sets in before the positive inotropic effect.

The effect of digitalis on the resting electrocardiogram (ECG) of healthy persons had been observed early on (Cohn & Fraser 1913 Pardee 1923 Routier & Puddu 1935 Larsen & al 1937 Geiger et al 1941 Dönhardt 1951 Koelbing 1950 Tiliakos 1953 and others) It has been constantly suggested that the ECG changes might be an indicator of the degree of digitalization.

The value of electrocardiography in conjunction with muscular work for the diagnosis of ischaemic heart disease is well documented. In Sweden stress has been placed on the importance of using a form of exercise and location of the electrodes which allow electrocardiography also during exercise (Bengtsson 1956 a, Sandberg 1957 and 1961 I Åstrand 1960) This increases the safety of the test for the patient and the accuracy of the electrocardiographic diagnosis.

It is imperative that different sources of error in the exercise test should be analysed in order that the diagnosis may be as certain as possible. Pharmacological preparations, especially the digitalis glucosides constitute an important source of error

Very little information has been published concerning the electrocardiographic reaction to digitalis during exercise.

Zwillinger (1935) found changes in the ST and T segments after exercise also in healthy persons who had received digitalis. Liebow & Feil (1941) studied the electrocardiographic reaction after exercise on 14 young healthy subjects. Digitalis was administered per os until signs of intoxication appeared Apart from lower heart rate and isolated cases of arrhythmia, a general finding was a depression of the ST segment and the authors maintain that it is impossible to distinguish the digitalis effect from the effect seen in coronary disease.

The effect of digitalis on the ECG during exercise has earlier been studied only by Yu et al. (1952) who injected digoxin

O_2 consumption

The O_2 consumption was calculated after analysis of the content of O_2 and CO_2 in the expired air ad modum Krogh-Haldane. The standard error of a single determination calculated from duplicate determinations and expressed as coefficient of variation was 0.6 % of the mean for CO_2 and 0 13 % for O_2 (n = 200)

The error of the method for the ventilation determined on separate days was at rest about 15 % and at the highest loads about 7 %. For duplicate determinations at the same examination the error of the method was at rest 5 % and during exercise 10 %. These values are of the same order of magnitude as reported earlier (cf I Åstrand, 1960 and Holmgren and Pernow 1958)

Mechanical efficiency

The O_2 uptake was measured at rest and was used for calculation of the mechanical efficiency. The mechanical efficiency was also calculated from predicted value (cf I Åstrand 1960) The two values of mechanical efficiency are compared in the account of the results. A caloric coefficient for oxygen of 4.825 cal/lit was used at rest and of 4.900 during exercise.

Lactate

Samples for lactate determination were collected from the fingertip at rest, at the 6th minute at each load, and 2 minutes after the end of exercise. The blood was precipitated with trichloracetic acid within 30 seconds. The analyses were made by Ström modification (1949) of the method of Barker and Summerson (1941) The error of the method, calculated from duplicate determinations on the same blood sample was 4 4 % at a mean concentration of 1 42 mM/l 2.1 % at mean concentration of 6.5 mM/l and 2.2 % at a mean concentration of 9.5 mM/l The error of the analysis was of the same order of magnitude as reported by Holmgren, (1956) Carlson and Pernow (1959) and I. Åstrand (1960) This method of accounting for the error of the method, accordingly does not reveal the sampling error The error of the method is considerably larger if duplicate determinations are made on the same individual on different days and at separat examinations.

Total amount of haemoglobin

The total haemoglobin was determined by the alveolar carbon dioxide method originally devised by Sjöstrand (1948) and modified in certain respects by Wiklander (1956) The standard error of the single determination calculated from duplicate determinations was 4 % at this laboratory The haemoglobin concentration, Hb (g/100 ml) was determined by haemolysing blood in 0.04 % ammonia. The density of the solution was measured in Beckman B spectrophotometer Blood samples were collected by pricking of the fingertip. The error of the method was about 4 % including the sampling error

The blood volume was calculated from total haemoglobin and haemoglobin concentration.

Heart volume

The heart volume was determined in prone position by the method of Larsson and Kjellberg (1948) and Kjellberg et al. (1949) with exposure in two planes without special regard for the contraction phases of the heart. Kjellberg et al. (1951) showed that the effect of the heart cycle on heart size in the recumbent body position is negligible except possibly when the pulse rate is very low By this method the posteroanterior projection is taken with

MATERIAL and METHODS

Material

A total of 92 tests were made on 64 healthy volunteers 32 men aged 17—55 years, average 28 years, and 32 women aged 20—55 years, average 35 years. They were mostly physicians, medical students and nurses. None had a history of cardiac or vascular disease and none exhibited any sign of illness at the clinical examination. All were in full time employment

Methods

Electrocardiography

Electrocardiograms (ECG) were recorded with a direct writing four-channel apparatus (Mingograph 42 Elema, Stockholm) The paper speed was 50 mm per second. The electrodes were plates of 30 mm diameter Eight leads were used for recordings at rest, in standing and after exercise viz. standard leads I II III and CR leads 1 2, 4 5 and 7 During exercise the indifferent electrode was moved to the forehead the CH lead. In normal cases CH and CR leads may be considered practically identical (Bengtsson 1956 c, Holmgren and Strandell 1961) The sensitivity was 10 mm for 1 mV

Orthostatic test

The exercise test was routinely preceded by an orthostatic test (Sandberg 1957 1961 Holmgren et al. 1957 and 1959) The subjects stood for 8 minutes as relaxed as possible with the head leaning against a wall, after which the ECG was recorded.

Exercise test

The exercise test was performed according to the method elaborated by Sjöstrand (1947) Wahlund (1948) Holmgren (1956) and others. The work was performed at successively rising loads on an electrically braked bicycle ergometer designed by Holmgren and Mattsson (1954) The subjects cycled for 6 minutes at each load starting at 300 kpm/min, then 600 900 and possibly 1200 for men, and 200 400 600 800 kpm/min for women. ECG records were taken during the test at the 2nd, 4th and 6th minute at each load, and immediately 3 minutes and 10 minutes after the end of the exercise. The same bicycle ergometer was used throughout the experimental series. It was calibrated annually but no major deviations were noted. The test was as a rule continued up to a heart rate of about 170 beats a minute (Robbe and Ström 1958, Hellström 1961 and others)

All heart rates were counted from the ECG tracings. The respiratory rate was counted by auscultation at rest, during the 5th minute at each load and after the completion of exercise. The working capacity (W_{170}) has been defined as the work performed at heart rate 170 beats/min.

Ventilation

The total ventilation was determined by collection of the expired air in a Douglas bag for 5 minutes at rest and during the 3rd — 5th minutes at each load. The gas volumes were measured in a gasometer The breathing rate was counted for 1 minute in the middle of each collecting period

Through the choice of lanatoside C, digitoxin and digoxin the study has comprised one compound with rapid effect and good solubility *(lanatoside C)* one with prolonged cumulative effect *(digitoxin)* and one peroral compound with rapid resorption capacity and brief effect *(digoxin)*

The drugs were given both in therapeutic and in toxic doses. Thus *lanatoside C* was given in doses of 0.4 and 0.8 mg intravenously and of 1 6 mg intravenously the latter may be regarded as an average digitalization dose. This dosage implies that some subjects become fully digitalized, others not. In the case of *digoxin* a single dose of 1 25 mg per os was used, which is the average digitalization dose for this glucoside. In the case of *digitoxin* doses which produced signs of intoxication in more than half of the subjects (1.2 — 3 6 mg) were used. These doses of digitalis are largely in accordance with those proposed by previous authors.

Procedure

A clinical evaluation of the subjects was made before the digitalis tests. This evaluation included in all cases medical history clinical examination, determination of haemoglobin concentration, sedimentation rate, and urinary examination for protein, sugar and casts. X-rays were taken of heart and lungs, and total haemoglobin was determined. ECG records were taken resting, standing, and during and after exercise. In the older age group determinations were made also of serum cholesterol, in some cases, too, of serum iron.

The subjects were not in basal state and ll took a light morning meal on the days when the tests were made All exercising electrocardiograms were made t the same time of the day about 2 hours after a light meal.

1 *Tests with lanatoside C*

a) *1.6 mg intravenously*

All digitalization was done in the morning A resting ECG was recorded after about 15 minutes of rest and lanatoside C was then slowly injected intravenously The injection generally took 3 minutes. The total dose was 1 6 mg, which was generally divided into two doses of 0.8 mg injected with half-an-hour's interval. This was done because some subjects who were given a single dose of 1.6 mg had pain in the arm even if the injection was given slowly No inconvenience was suffered when the dose was divided. After the injection, electrocardiograms were recorded at rest at 15-minute intervals for about 1 hour About 3 hours after the last injection the exercise test was started Thereafter a heart X-ray was taken, followed by determination of total haemoglobin. The subject returned daily to the laboratory always at the same time in the afternoon, and daily ECG records were taken resting, standing, during and after exercise as long as ECG changes persisted. This group comprised 21 men aged 22 — 55 years, average 29 years, and 21 women aged 21 — 54 years, average 32 years.

b) *0 4 and 0.8 mg lanatoside C intravenously*

The procedure was largely the same as for the 1.6 mg test. The subjects were injected with 0 4 mg lanatoside C intravenously in the morning. Three hours later ECG was recorded resting, standing, and during and after exercise. The same procedure was repeated a week later this time after injection of 0 8 mg lanatoside C intravenously This group comprised 9 subjects, 6 men aged 17 — 33 years, aver

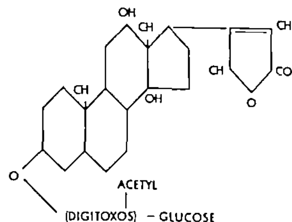


Fig 1

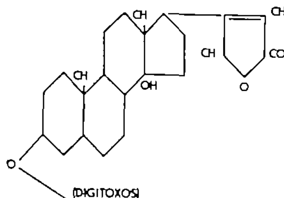


Fig 2.

the tube angled 30° The error of the method according to Kjellberg *et al.* (1951) is about 4 % The method used in the present study differs from the abovementioned in the respect that we did not use an angled tube and the heart volume was calculated according to Jonsell (1939) By the method of heart size determination in recumbent position employed at the Serafimer Hospital the error is about 4 % The heart volume was also determined in standing position.

Blood pressure

The blood pressure was measured in the right arm recumbent at heart level and after 8 minutes standing by the auscultatory cuff method. The diastolic pressure was taken to be the pressure when the Korotkoff sounds disappeared.

Choice of digitalis drugs

Cardioactive glucosides is the collective name for a group of substances in nature with the same basic properties in respect of effect and chemical structure At present there is no synthetic digitalis compound. The pharmacological effect of a cardiac glucoside lies in the aglucone, while properties such as solubility duration of effect, etc. lie in a sugar molecule, *usually a monosaccharide.*

Digitalis drugs with varying time of onset and duration of effect were chosen for this study They have also been thoroughly investigated in respect of their pharmacological and clinical effects and of their resorption and excretion conditions. They have all been put to wide clinical use.

Lanatoside C (Cedilanid(R) Sandoz) is one of the three glucosides that has been isolated in *digitalis lanata* The aglucone in lanatoside C consists of digoxigenin the molecule also contains three molecules of digitoxose, one molecule of glucose and one molecule of acetic acid (fig 1) Cedilanid has been injected intravenously

Digitoxin (ACO) has been isolated both from *digitalis lanata* and *digitalis purpurea.* The cardiactive aglucone consists of digitoxigenin with three molecules of digitoxose, glucose and acetyl as radicals (fig 2) Digitoxin has been given perorally

Digoxin (Lanacrist Draco) is in principle the same cardioactive glucoside as lanatoside C, but with one glucose molecule and one acetyl group less. Digoxin may thus be regarded as the pure glucoside from lanatoside C. Digoxin has been given perorally

Subjective symptoms

General considerations

A large variety of subjective symptoms in conjunction with digitalis medication have been described in the literature. The symptoms noted have been *gastrointestinal* such as a feeling of discomfort in the epigastrium, increased salivation, anorexia, nausea, vomiting and diarrhoea *neurological* such as headache, visual disorders (scotoma, yellow-green vision, double vision) *general* symptoms such as fatigue perspiration *cardiac* symptoms such as palpitation (actually a feeling that the heart is beating hard) and symptoms due to disorders of impulse formation and conduction disorders (Withering 1785 Herrmann et al., 1944 Burwell et al., 1950, Cohen, 1952 a, b, and others)

Rarer symptom are objectively observable endocrinological disorders such as gynaecomastia (Le Winn 1953) The gastrointestinal symptoms are by far the most common and occur not only in response to galenicals but also to purified drugs.

The best early description of the appearance and course of these symptoms is that by Withering (1785) who wrote

> The Foxglove when given in very large and quickly-repeated doses, occasions sickness, vomiting, purging, giddiness, confused vision, objects appearing green or yellow increased secretion of urine, with frequent motions to part with it, and sometimes inability to retain it slow pulse, even as slow as 35 in a minute cold sweats, convulsions, syncope, death.
>
> When given in a less violent manner it produces most of these effects in a lower degree

Subjective symptoms are considered to constitute a certain measure of digitalization, from "full digitalization" to intoxication. Toxic signs are therefore regarded as extremes of the therapeutic effect (Wright 1960)

A. *Symptoms following intravenous injection of lanatoside C*

Nine subjects were given 0.4 mg of lanatoside C intravenously None had subjective symptoms.

Eight subjects were given a dose of 0.8 mg intravenously One subject had nausea and became tired about two hours after the injection. The symptoms lasted about half-an-hour Otherwise no subjective symptoms appeared.

Forty-two subjects were injected with 1.6 mg of lanatoside C, 12 of whom developed subjective symptoms (table 1 A) The symptoms generally appeared 1½—2 hours after the injection. All were able to perform an exercise test although one (no. 27) broke off the test at a lower load than before the medication. The symptoms were comparatively mild and of brief duration. The nature of the symptoms is shown in table 1 One subject (no. 27) had more pronounced symptoms lasting several hours. It will be seen from the table that the dose per kg body weight varied from 0.018 to 0.031 mg. The mean dose 0.025 mg/kg bodyweight was the same as for those who had no symptoms. The dose per kg body weight was therefore of no significance. Almost all subjects who were given 1.6 mg lanatoside C thought that they perspired more during the exercise test, but only the definite cases are included in the table.

age 22 years, and 3 women aged 20 — 51 years, average 33 years.

2 *Digitoxin per os*

Digitoxin was given per os for two to ten days in a total dose of 1 2 — 3 6 mg On the appearance of nausea or serious ECG changes, the medication was stopped With a few exceptions the digitoxin tablets were administered daily in the laboratory in order to ensure that the subjects actually took them. During the digitalization resting electrocardiograms were recorded daily On the last day or one day after the digitalization the procedure was repeated with exercise test and determination of heart volume and total haemoglobin. In ten cases determinations were made also of the lung ventilation, oxygen consumption and of the lactate concentration during and after exercise, all in the same order as in the tests with 1 6 mg of lanatoside C intravenously

After the course of digitalization, ECG at rest, standing and in conjunction with exercise were recorded once a week as long as ECG changes persisted This group comprised 23 subjects 11 men aged 22—55 years, average 30 years, and 12 women aged 21—55 years, average 36 years.

3 *Digoxin per os*

A single dose of 1.25 mg digoxin (Lanacrist(R) was given per os in the laboratory in the morning after a light meal. The subjects kept in contact with the laboratory during the day for check of subjective symptoms etc. Five hours later orthostatic and exercise ECG were recorded. This procedure was repeated daily until ECG changes had disappeared. This group comprised ten subjects, 6 men aged 23—37 years, average 30 years, and 4 women aged 21—52 years, average 37 years.

Statistical methods

Conventional statistical methods have been used (Fisher 1950 Kemp & Nielsen 1959)

Significance was testaed by the t — test
Levels of significance
$p = 0.05—0.01$ probably significant
$p = 0.01—0.001$ significant
$p < 0.001$ highly significant

B. Symptoms following administration of digitoxin

This series comprised 25 subjects, 13 of whom developed subjective symptoms (table 1 B). The subjective symptoms after digitoxin were more pronounced than after lanatoside C intravenously. The total dose varied from 1.2 mg to 3.6 mg per os spread over 2—14 days. Here again, accordingly there was no correlation between dose and symptoms. The symptoms generally appeared 8—12 hours, on one occasion 3 hours, after the last dose. Their duration was 2—3 hours.

C. Symptoms following administration of digoxin

Ten subjects were given a single dose of 1.25 mg digoxin. Eight of them had subjective symptoms, as shown in table 1 C, in all cases consisting of nausea, in two combined with vomiting. The symptoms appeared 40 minutes to 2 hours after medication and were of comparatively brief duration. All subjects were able to carry out the exercise test.

Summary

The incidence and nature of the subjective symptoms in conjunction with digitalis medication in healthy volunteers is reported. The cardioactive glucosides were administered partly intravenously, partly per os.

Lanatoside C in doses of 0.4, 0.8 and 1.6 mg was used as parenteral glucoside. No subject developed symptoms after 0.4 mg, only one after 0.8 mg, and even after 1.6 mg the subjective symptoms were comparatively rare, of a mild nature and rapidly disappeared. In those who developed symptoms the treshold dose per kg body weight varied very greatly and did not differ from that administered to subjects who had no symptoms.

The subjective symptoms were more common, more pronounced and of longer duration after oral administration of digitoxin. The dose after which symptoms appeared again varied, from a total of 1.2 to 3.6 mg spread over 2—14 days. No subjective symptoms were reported after smaller doses than 1.2 mg.

After a single dose of 1.25 mg digoxin, symptoms were common and fairly pronounced, and appeared soon after the medication, as opposed to the other oral preparation, digitoxin. The symptoms rapidly disappeared.

Effect on heart rate

1. *Heart rate at rest*

A. *Lanatoside C*

The resting heart rate for men was 66 ± 2.5, S.D. 11 (n = 21) after injection of 1.6 mg of lanatoside C intravenously. The difference in rate before and after digitalis is not significant (p = 0.5—0.4) (table 5).

For women the corresponding values were 72±2.5, S.D. 12 (n = 21) before and 66±3.2, S.D. 15 (n = 21) after lanatoside C. Here the rate is rather lower and the difference probably significant (p = 0.05—0.01). If men and women are taken together there is no significant difference (p = 0.2—0.1).

Lanatoside C was given also in smaller doses, 0.4—0.8 mg. The heart rate before administration was 68 ± 1, S.D. ± 11 and after 65 ± 2.0, S.D. ± 8 (n = 17). The difference before and after these small doses of lanatoside C is not significant (p = 0.5—0.2).

B. *Digitoxin*

This series (table 6) comprised 23 subjects, of whom 11 men and 12 women, who received digitoxin tablets per os in

Table 1

A. *Subjective symptoms after single dose of 1.6 mg lanatoside C intravenously*

Subject number	Symptoms	Dose mg/kg body weight	Time of onset of symptoms
2	Nausea and fatigue	0.023	1 h
3	Feeling of discomfort and fatigue	0.025	1½ h
5	Fatigue	0.019	2 h
8	Nausea fatigue, perspiration	0.026	1 h
11	Feeling of discomfort in epigastrium	0.025	1½ h
15	Feeling of discomfort in epigastrium, fatigue perspiration	0.018	2 h
19	Fatigue	0.029	1 h
20	Feeling of discomfort in epigastrium	0.031	1 h
25	Fatigu	0.020	2 h
27	Nausea, vomiting fatigue perspiration, dizziness	0.029	1½ h
31	Nausea, fatigue	0.031	2 h
40	Fatigue	0.028	1 h

B. *Subjectic symptoms fter digitoxin per s*

Subject number	Symptoms	Dose mg	Approx. time in hours after last dose
11	Increased salivation	1.2	8 h
31	Nausea, omiting palpitation, fatigue	1.6	12 h
32	Nausea	1.6	6 h
34	Nausea, fatigue	1.2	8 h
37	Nausea, increased salivation, palpitation	1.8	ca 12 h
38	Fatigue	2.6	5—6 h
41	Palpitation, nausea	1.6	12 h
42	Anorexia, nausea	1..	3 h
43	Palpitation, perspiration, epigastrial discomfort, fatigue	1.6	12 h
45	Epigastrial discomfort, fatigue	2.0	10—12 h
46	Nausea, persp ration, palpitation	3.6	10 h
47	Nausea, epigastrial discomfort, f tigue palpitation	1.4	8—10 h
55	F tigue	2.8	

C. *Subj ctive symptoms fte single dose f 1.25 mg digoxin per os*

Subject number	Symptoms	Dose mg/kg	Time of onset of symptoms
15	Nausea, vomiting	0.015	1 h
24		0.022	40 min.
35		0.016	1 h
50		0.022	1 h
54		0.017	1 h
56		0.020	1½ h
57	vomiting	0.017	2 h
51		0.019	1 h

To throw furher light on this situation, further experiments were made in which 1.6 mg of lanatoside C intravenously and digitoxin orally were given to 14 subjects on different occasions.

These show the same pattern, a resting heart rate of 65 before digitalization, of 63 beats/min after lanatoside C and 63 beats/min likewise after digitoxin (table 3). The heart rate in standing was also higher after lanatoside C. The difference is probably significant ($p = 0.0?–0.01$). The orthostatic pulse reaction, i.e. the difference between recumbent and standing heart rate, increased after lanatoside C, the difference being significant ($p = 0.01–0.001$).

After digitoxin, on the other hand, there was no significant difference either at rest ($p = 0.4–0.3$), standing ($p = 0.9–0.8$) or in respect of orthostatic pulse reaction ($p = 0.4–0.3$).

In the lanatoside C and digitoxin groups there were subjects who had subjective symptoms of varying degree, and it is not unreasonable to imagine that they represent roughly the same degree of digitalization.

It is apparent from table 4 that the tendency is the same as before. The resting heart rates are lower for those who had subjective symptoms after digitalization, whether with lanatoside C or digitoxin. In both groups the difference was probably significant ($p = 0.05–0.01$).

In the lanatoside C group without symptoms the orthostatic pulse reaction was 23 beats/min after digitalization against 15 before; the difference in reaction is significant ($p = 0.01–0.001$) and in the group with symptoms the reaction was still stronger.

Before lanatoside C the orthostatic pulse reaction was 17 and after 33 beats/min ($p < 0.001$) in this latter group.

In the symptomatic group the standing heart rate before lanatoside C was 88 beats/min, after digitalization 99 beats/min. The difference is significant ($p = 0.01–0.001$).

The subjects who had signs of intoxication after digitoxin had a rather lower resting rate (table 4), the difference being probably significant ($p = 0.02–0.01$). But there were no significant differences in respect of standing heart rate and orthostatic pulse reaction.

3 Heart rate during exercise

A. Lanatoside C

The results are shown in table 5. It will be seen that the heart rate reactions during exercise before and after intravenous injection of lanatoside C were practically identical. At a load of 300 kpm/min the men had an average heart rate of 98 beats/min before and 99 after digitalization.

At the next load 600 kpm/min the corresponding heart rates were 126 and 127 beats/min. At 900 kpm/min the average rate was identical before and after lanatoside C, 157 beats/min, and likewise at 1200 kpm/min, 168 beats/min.

The women, whose resting heart rate was lower after digitalization, displayed no appreciable changes during exercise. At a load of 200 kpm/min their average heart rate was 107 beats/min before and 108 after lanatoside C. At a load of 400 kpm/min the mean values were, respectively 134 and 135 beats/min, at 600 kpm/min they were identical before and after: 160 beats/min, respectively. Intravenous lanatoside C caused no significant change in heart rate during exercise.

B. Digitoxin

The pulse reaction during exercise before and after digitoxin is shown in table 6.

doses varying between 1 2 mg spread over two days and 3 6 mg spread over 14 days. For men the *heart rate before digitoxin* was 67 ± 2.9 S.D 10 (n = 11) and after digitoxin 64 ± 3.5 S D 12 (n = 11) i.e very slightly lower The difference was not significant (p = 0.3—0 2) For women the *corresponding* values were 66 ± 3.2, S D ± 11 (n = 12) before and 62 ± 3 0 S D 10 (n = 12) after Here again the difference is not significant (p = 0.2—0 1)

C. *Digoxin*

After a single dose of 1 25 mg of digoxin *per os to 10 subjects (6 men and 4 women)* the heart rate before administration was 68±3 6 S.D ±11 (n = 10) and after 65±3.5 S.D ±11 (n = 10) The difference is not significant (p = 0 4—0 3)

2 *The orthostatic reaction*

A. *Lanatoside C*

The orthostatic pulse reaction (beats/ *min*) i.e *the difference in heart rate* between recumbent and standing position, was 14 0 ± 1.5 S D ± 7 4 (n = 42) before intravenous injection of 1 6 mg of lanatoside C and ?5 8 ± 2 3 S.D 15 (n = 42) after (table 2) Digitalization by lanatoside C thus brought a marked orthostatic reaction, *the difference* being highly significant (p <0 001)

A similar result was obtained with the lower doses of lanatoside C. Before digitalization the orthostatic reaction was *16.3±3 2 S.D 10 The corresponding* values after digitalization were 25.3±2 8 S.D 11 4 Again the difference is highly significant (p <0 001) (n = 17)

B *Digitoxin*

The orthostatic pulse reaction before digitoxin was 16.3±2 S.D ±10 (n = 23) and after 15 7±1.9 *beats/min* S D ± 9 0 There is thus no difference before and after digitoxin per os (p = 0 6—0.5)

C. *Digoxin*

In the digoxin group the orthostatic pulse reaction before medication was 12 9±1 7 S.D ±5 4 beats/min and after a single oral dose of 1 25 mg 14 6±3.5 S.D 11 Here again there is no difference (p = *0 6—0 5*)

Comparison between different groups

Table 2 contains data of the entire material before digitalization and after different doses of lanatoside C and digitoxin.

At rest the lowest values of heart rate were obtained after digitoxin. The difference is fairly small however about 5 *beats/min, though probably significant* (p = 0 05—0 02) The other drugs showed no significant difference compared with the predigitalization figures (p = 0 6—0.5) Considering the heart rates in *standing* the lanatoside C *groups* differ from the others, i.e. the standing rate is higher about *90 beats/min* compared with 78 after digitoxin and 79 after digoxin. Before administration the heart rate was 83 beats/min

The rise in heart rate after lanatoside C *is probably significant* (*p* = 0 05—0 01) after the two other glucosides there was no significant *difference* in standing *position*. The orthostatic pulse reaction is most affected after lanatoside C as had appeared earlier the difference is highly significant (p <0.001) There is undeniably *significant difference in* standing position. C in the standing position than to digitoxin and digoxin.

To throw furher light on this situation, further experiments were made in which 1.6 mg of lanatoside C intravenously and digitoxin orally were given to 14 subjects on different occasions.

These show the same pattern, a resting heart rate of 65 before digitalization, of 63 beats/min after lanatoside C and 63 beats/min likewise after digitoxin (table 3). The heart rate in standing was also higher after lanatoside C. The difference is probably significant ($p = 0.02—0.01$). The orthostatic pulse reaction, i.e. the difference between recumbent and standing heart rate, increased after lanatoside C, the difference being significant ($p = 0.01—0.001$).

After digitoxin, on the other hand, there was no significant difference either at rest ($p = 0.4—0.3$), standing ($p = 0.9—0.8$) or in respect of orthostatic pulse reaction ($p = 0.4—0.3$).

In the lanatoside C and digitoxin groups there were subjects who had subjective symptoms of varying degree, and it is not unreasonable to imagine that they represent roughly the same degree of digitalization.

It is apparent from table 4 that the tendency is the same as before. The resting heart rates are lower for those who had subjective symptoms after digitalization, whether with lanatoside C or digitoxin. In both groups the difference was probably significant ($p = 0.05—0.01$).

In the lanatoside C group without symptoms the orthostatic pulse reaction was 23 beats/min after digitalization against 13 before; the difference in reaction is significant ($p = 0.01—0.001$) and in the group with symptoms the reaction was still stronger.

Before lanatoside C the orthostatic pulse reaction was 17 and after 33 beats/min ($p < 0.001$) in this latter group.

In the symptomatic group the standing heart rate before lanatoside C was 88 beats/min, after digitalization 99 beats/min. The difference is significant ($p = 0.01—0.001$).

The subjects who had signs of intoxication after digitoxin had a rather lower resting rate (table 4), the difference being probably significant ($p = 0.02—0.01$). But there were no significant differences in respect of standing heart rate and orthostatic pulse reaction.

3. Heart rate during exercise

A. Lanatoside C

The results are shown in table 5. It will be seen that the heart rate reactions during exercise before and after intravenous injection of lanatoside C were practically identical. At a load of 300 kpm/min the men had an average heart rate of 98 beats/min before and 99 after digitalization.

At the next load 600 kpm/min the corresponding heart rates were 126 and 127 beats/min. At 900 kpm/min the average rate was identical before and after lanatoside C, 157 beats/min, and likewise at 1200 kpm/min, 168 beats/min.

The women, whose resting heart rate was lower after digitalization, displayed no appreciable changes during exercise. At a load of 200 kpm/min their average heart rate was 107 beats/min before and 108 after lanatoside C. At a load of 400 kpm/min the mean values were, respectively 134 and 133 beats/min, at 600 kpm/min they were identical before and after 160 beats/min, respectively. Intravenous lanatoside C caused no significant change in heart rate during exercise.

B. Digitoxin

The pulse reaction during exercise before and after digitoxin is shown in table 6.

doses varying between 1 2 mg spread over two days and 3 6 mg spread over 14 days. For men the heart rate before digitoxin was 67 ± 2 9 S.D 10 (n = 11) and after digitoxin 64 ± 3.5 S.D 12 (n = 11) i.e. very slightly lower The difference was not significant (p = 0.3—0 2) For women the corresponding values were 66 ± 3 2 S.D ± 11 (n = 12) before and 62 ± 3 0 S.D 10 (n = 12) after Here again the difference is not significant (p = 0 2—0 1)

C. *Digoxin*

After a single dose of 1 25 mg of digoxin per os to 10 subjects (6 men and 4 women) the heart rate before administration was 68±3 6, S.D ±11 (n = 10) and after 65±3.5 S.D ±11 (n = 10) The difference is not significant (p = 0 4—0 3)

2. *The orthostatic reaction*

A. *Lanatoside C*

The orthostatic pulse reaction (beats/min) i.e. the difference in heart rate between recumbent and standing position, was 14 0 ± 1.5 S.D ± 7 4 (n = 42) before intravenous injection of 1 6 mg of lanatoside C and 25 8 ± 2.3 S.D 15 (n = 42) after (table 2) Digitalization by lanatoside C thus brought a marked orthostatic reaction, the difference being highly significant (p <0 001)

A similar result was obtained with the lower doses of lanatoside C. Before digitalization the orthostatic reaction was 16 3±3 2, S.D 10 The corresponding values after digitalization were 25.3±2.8 S.D 11 4 Again the difference is highly significant (p <0 001) (n = 17)

B *Digitoxin*

The orthostatic pulse reaction before digitoxin was 16.3±2, S.D ±10 (n = 23) and after 15 7±1.9 beats/min, S.D ± 9 0 There is thus no difference before and after digitoxin per os (p = 0 6—0.5)

C. *Digoxin*

In the digoxin group the orthostatic pulse reaction before medication was 12.9±1 7 S.D ±5 4 beats/min and after a single oral dose of 1.25 mg 14 6±3.5 S.D 11 Here again there is no difference (p = 0 6—0.5)

Comparison between different groups

Table 2 contains data of the entire material before digitalization and after different doses of lanatoside C and digitoxin

At rest the lowest values of heart rate were obtained after digitoxin. The difference is fairly small, however about 5 beats/min, though probably significant (p = 0 05—0 02) The other drugs showed no significant difference compared with the predigitalization figures (p = 0 6—0.5) Considering the heart rates in standing the lanatoside C groups differ from the others, i.e. the standing rate is higher about 90 beats/min, compared with 78 after digitoxin and 79 after digoxin. Before administration the heart rate was 83 beats/min.

The rise in heart rate after lanatoside C is probably significant (p = 0 05—0.01) after the two other glucosides there was no significant difference in standing position. The orthostatic pulse reaction is most affected after lanatoside C, as had appeared earlier the difference is highly significant (p <0 001) There is undeniably significant difference in standing position. C in the standing position than to digitoxin and digoxin.

Table 2 Heart rate at rest 8 minutes standing and differ nc betw en rest and st nding, in beats/min. (M ± S.E., S.D.) The effect of different gl osides.

		rest	standing	orthostatic pulse reaction
Before digitalization	69	68 ± 1.3 11	83 ± 1.7 14	14.4 ± 1.0 7.9
After lanatoside C, 1.6 mg	42	65 ± 2.0 13	91 ± 3.1 12	25.8 ± ..3 15.1
After lanatoside C 0.4—0.8 mg	17	65 ± 2.0 8	90 ± 3.4 14	23.3 ± 2.8 11.4
After digitoxin	23	65 ± 2.3 11	78 ± 3.0 14	13.7 ± 1.9 9.0
After digoxin	10	65 ± 3.5 11	79 ± 5.2 17	14.6 ± 3.5 11.0

Table 3 Heart rate at rest 8 minutes standing and differenc between est and standing, in beats/min. (M ± S.E., S.D.) Comparison between the effect of lanatosid C and digitoxin on same subjects

		rest	standing	orthostatic pulse reaction
Befor digitalization	14	64 ± 2.9 11	79 ± 3.7 13.8	14.4 ± 2.3 8.6
After lanatoside C, 1.6 mg	14	63 ± 4.2 16	86 ± 4.6 17	22.3 ± 3.6 13.5
After digitoxin	14	63 ± 3.2 12	80 ± 4.0 15	16.9 ± 2.3 8.8

(women) before digitoxin and, respectively 115 and 107 after. The differences are not significant (p = 0.6—0.4 and 0.2—0.1).

Nor was there any difference 3 minutes after exercise between the values before and after administration of digitoxin.

C. Digoxin

The same remarks apply to the heart rate reaction after exercise in conjunction with digoxin medication as with lanatoside C and digitoxin.

Before digoxin the heart rate was 107±S.D. 16 beats/min (n = 10) immediately after exercise, after digoxin 104±S.D. 16; the difference is not significant (p = 0.3—0.2). Three minutes after exercise the figures were, respectively 92±S.D. 8 and 90±S.D. 7, again without significant difference. (In the digoxin group men and women are presented together.)

At a load of 300 kpm/min the men's heart rate was 100 beats/min before and 96 after digitoxin. The difference is not significant ($p = 0.2—0.1$).

At 600 kpm/min the corresponding figures were 129 and 124 beats/min, again being non-significant ($p = 0.1—0.05$).

At 900 kpm/min the heart rate was 160 beats/min before and 153 beats/min, after digitoxin. The difference being probably significant ($p = 0.02—0.01$).

At the highest load of 1200 kpm/min the rates were respectively 169 and 167 beats/min, the difference being nonsignificant.

For women the tendency was the same as for men. At 200 kpm/min the rate was 95 beats/min before and 92 after digitalis the difference is not significant ($p = 0.02—0.1$). At 400 kpm/min the figures were 121 and 120 again no difference ($p = 0.8—0.7$). At 600 kpm/min they were 156 and 154 ($p = 0.5—0.4$) at 800 kpm/min 170 and 165 the difference being probably significant ($p = 0.02—0.01$). Only six subjects, however exercised at this last load.

C. *Digoxin*

The subjects who were given digoxin per os were comparatively few altogether 10 of whom 6 men and 4 women. Since men and women exercised at different loads, they are recorded separately and the material is judged to be too small for statistical analysis.

At a load of 300 kpm/min the average heart rate for men before digoxin was 91 beats/min (range 66—106) and 90 after (range 68—118).

At 600 kpm/min the figures were 121 (range 90—136) before and 117 (range 92—152) after digoxin, at 900 kpm/min 149 (range 122—168) before and 142 (range 92—152) after.

For women the rate at 200 kpm/min before digoxin was 96 beats/min (range 86—106) and 100 after (range 84—120). At 400 kpm/min the figures were 119 (range 110—130) before and 118 (range 100—136) after and at 600 kpm/min 155 (range 136—164) before and 150 (range 128—168) after digoxin.

The heart rate reaction during exercise showed the same tendency after digoxin per os as after digitoxin.

4. *Heart rate after exercise*

The heart rate immediately and 3 minutes after the end of exercise is illustrated in tables 5 and 6.

There was no difference before and after digitalization either with lanatoside C, digitoxin or digoxin. Men and women had the same pulse reaction after exercise.

A. *Lanatoside C*

The men's heart rate immediately after the end of exercise was 108 beats/min before and 109 after digitalization (table 5). The corresponding figures for women were 113 and 108 beats/min, the difference before and after digitalization being nonsignificant ($p = 0.?—0.1$). Three minutes after the end of exercise the figures for nondigitalized subjects were 96 beats/min (men) and 94 beats/min (women) after digitalization 94 and 92. In neither case was the difference significant ($p = 0.4—0.2$).

B. *Digitoxin*

In the case of digitoxin as well the heart rate after exercise was little affected in relation to the situation before digitalization.

Immediately after exercise the heart rates were 111 beats/min (men) and 114

Table 2. Heart rate at rest, 8 minutes standing and difference between rest and standing in beats/min. (M ± S.E., S.D.) The effect of different glucosides

	n	rest	standing	orthostatic pulse reaction
Before digitalization	69	68 ± 1.3 11	83 ± 1.7 14	14.4 ± 1.0 7.9
After lanatoside C, 1.6 mg	42	63 ± 2.0 13	91 ± 3.1 12	23.8 ± ?.5 15.1
After lanatoside C 0.4—0.8 mg	17	65 ± 2.0 8	90 ± 3.4 14	25.5 ± 2.8 11.4
After digitoxin	23	63 ± 2.5 11	78 ± 3.0 14	15.7 ± 1.9 9.0
After digoxin	10	65 ± 3.5 11	79 ± 5.2 17	14.6 ± 3.5 11.0

Table 3. Heart rate at rest, at 8 minutes standing and difference between rest and standing in beats/min. (M ± S.E., S.D.) Comparison between the effect of lanatoside C and digitoxin on same subject

		rest	standing	orthostatic pulse reaction
Before digitalization	14	64 ± 2.9 11	79 ± 3.7 13.8	14.4 ± 2.3 8.6
After lanatoside C 1.6 mg	14	63 ± 4.2 16	86 ± 4.6 17	22.5 ± 3.6 13.3
After digitoxin	14	63 ± 3.2 12	80 ± 4.0 15	16.9 ± 2.3 8.8

(women) before digitoxin and, respectively 113 and 107 after. The differences are not significant (p = 0.6—0.4 and 0.2—0.1).

Nor was there any difference 3 minutes after exercise between the values before and after administration of digitoxin.

C. Digoxin

The same remarks apply to the heart rate reaction after exercise in conjunction with digoxin medication as with lanatoside C and digitoxin.

Before digoxin the heart rate was 107± S.D. 16 beats/min (n = 10) immediately after exercise, after digoxin 104±S.D. 16; the difference is not significant (p = 0.3—0.2). Three minutes after exercise the figures were, respectively 92±S.D. 8 and 90±S.D. 7, again without significant difference. (In the digoxin group men and women are presented together.)

Table 4 Heart rate at rest, 8 minutes standing and difference between rest and standing (M ± S.E., S.D.) in beats/min.

	n	rest	standing	orthostatic pulse reaction
Before lanatoside C *no symptoms*	30	68 ± 2.1 11	81 ± 2.8 15	13.3 ± 1.3 7
After lanatoside C *no symptoms*	30	64 ± 2.5 14	87 ± 3.5 19.2	23 0 ± 1.8 10
Before lanatoside C, symptoms	12	71 ± 3 4 17	88 ± 4.2 15	17.3 ± 2.2 8
After lanatoside C, symptoms	12	66 ± 3 4 12	99 ± 3 7 20	32.9 ± 3 2 12
Before digitoxin, no symptoms	10	67 ± 3 9 12	77 ± 3.9 12	9.3 ± 1 6 5
After digitoxin no symptoms	10	67 ± 3 7 12	80 ± 4.3 14	12.7 ± 1.9 6
Before digitoxin, symptoms	13	66 ± 2.4 9	81 ± 3.5 13	15.5 ± 1.9 7
After digitoxin, symptoms	13	59 ± 2.6 9	77 ± 4.3 15	18.0 ± 2.2 8

Effect on certain aspects of circulatory function and dimensions

1. *Working capacity at a heart rate of 170 beats/min (W_{170})*

In view of the linear relationship between load and heart rate (Wahlund 1948) and the fact, as noted previously, that the heart rate was almost identical at comparable loads before and after digitalization, it is natural that no appreciable difference in values of W_{170} appeared as a result of the method of digitalization.

A. *Lanatoside C*

W_{170} was, for men, 1055 ± 42 kpm/min (S.D ± 192) before and 1038 ± 41 kpm/min (S.D ± 189) after administration of lanatoside C intravenously (n = 21). The difference is not significant (p = 0.2—0.1). For women the figures were 615 ± 39 kpm/min before and 640 ± 27 after digitalization (n = 21). Again there was no significant difference before and after lanatoside C (p = 0.1—0.05).

B. *Digitoxin*

In this group the figures of W_{170} for men were 1087 ± 69 kpm/min (S.D ± 230) before and 1130 ± 67 (S.D ± 224) after digitalization (n = 11); the difference is not significant (p = 0.1—0.05).

The corresponding figures for women in this group were 695 ± 40 and 714 ± 36 kpm/min, respectively (n = 12). Again there was no significant differences before and after digitoxin (p = 0.2—0.1).

Table 5 H art ate (beats/min.) befor nd after administration of l a i i d C (M ± S.E., S.D.)

Sex	Work load, kpm/min.	No. of subjects	Heart rate, beats/min	
			before	after
M	resting	21	66 ± 2.3 11	64 ± 2.8 13
	standing	21	79 ± 2.9 13	87 ± 3.6 17
	300	21	98 ± 2.8 13	99 ± 3.3 15
	600	21	126 ± 3.2 15	127 ± 3.5 16
	900	21	157 ± 3.6 17	157 ± 3.6 17
	1200	12	168 ± 1.9 7	168 ± 2.5 8
	immed. after work	21	108 ± 3.0 14	109 ± 4.0 19
	3 min after work	21	95 ± 1.9 9	96 ± 2.6 11
F	resting	21	72 ± 2.5 12	66 ± 3.2 15
	standing	21	87 ± 3.4 16	94 ± 4.8 22
	200	21	107 ± 3.0 14	108 ± 3.6 16
	400	21	134 ± 3.9 18	133 ± 3.6 16
	600	18	160 ± 3.6 15	160 ± 3.3 14
	800	7	170 ± 3.9 10	168 ± 2.1 8
	immed. after work	21	113 ± 4.0 19	108 ± 4.3 21
	3 min after work	21	94 ± 2.9 13	94 ± 3.4 16

Table 6 Heart rat (beats/min) before and after administration of d i g i t o x i n (M ± S.E., S.D)

Sex	Work load, kpm/min	No. of subjects	Heart rate, beats/min	
			before	after
M	resting	11	67 ± 2.9 10	64 ± 3.5 12
	standing	11	81 ± 2.9 10	81 ± 4.9 16
	300	11	100 ± 3.5 11	96 ± 3.8 12
	600	11	129 ± 4.5 15	124 ± 4.5 15
	900	11	160 ± 4.7 16	153 ± 5.1 17
	1200	6	169 ± 3.5 8	167 ± 3.8 9
	immed. after work	11	111 ± 4.7 16	113 ± 4.4 15
	3 min. after work	11	96 ± 2.6 9	94 ± 3.2 10
F	resting	12	66 ± 3.2 11	62 ± 3.0 10
	standing	12	78 ± 4.3 15	76 ± 3.7 13
	200	9	95 ± 4.6 14	92 ± 3.6 11
	400	11	121 ± 5.2 17	120 ± 4.5 15
	600	11	156 ± 4.7 15	154 ± 4.8 16
	800	6	170 ± 4.7 11	165 ± 4.3 11
	immed. after work	12	114 ± 4.4 15	107 ± 5.4 19
	3 min. after work	11	94 ± 3.4 11	92 ± 4.9 16

C. *Digoxin*

In this group the W_{170} for men was 1112 kpm/min (range 970—1315) before and 1074 (range 950—1320) after digitalization (n = 6).

The figures for women were 682 kpm/min (range 611—770) and 690 (range 620—760) respectively (n = 4). The results are entirely in accordance with the findings for lanatoside C and digitoxin.

The occurrence of subjective symptoms, i.e. signs of digitalis intoxication, caused no change in W_{170}.

In the lanatoside C group four men had subjective symptoms: their average W_{170} was 1003 kpm/min before digitalization and 994 after. Eight women had subjective symptoms after lanatoside C: their W_{170} was 619 before digitalization and 611 kpm/min after ($p = 0.8$—0.7).

In the digitoxin group six men had subjective symptoms: their W_{170} before digitalis was 1196 kpm/min before and 1218 kpm/min after ($p = 0.1$—0.05). For women in this group the figures were, respectively, 649 and 673 kpm/min (n = 7) ($p = 0.3$—0.2).

2. *Total amount of haemoglobin*

Total haemoglobin did not show any change after digitalization.

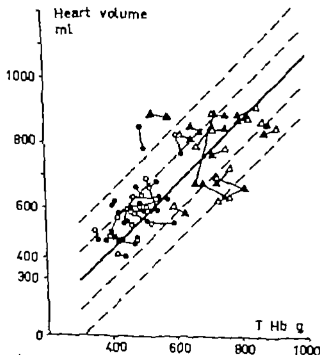


Fig. 3.

Heart volume in relation to total haemoglobin. The continuous line represents the normal regression line described by Holmgren et al. (1957). The dotted lines indicate ± standard deviation and twice the standard deviation.

Unfilled circles represent women before digitalization, filled circles after. Unfilled triangles represent men before digitalization, filled triangles after.

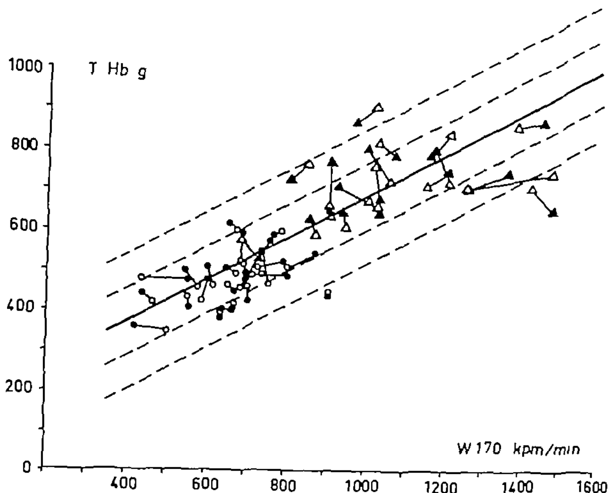


Fig 4 Total haemoglobin in relation to working capacity at pulse 170 W_{170}. Normal regression line and symbols as in fig. 3

Before 1 6 mg of lanatoside C intravenously the total haemoglobin for that group of male subjects was 712 ± 20 g (S.D ± 87) and 723 ± 20 g (S.D ± 82) after (n = 19) The difference is not significant (p = 0 6—0 5) For women the figures were 454 ± 9 and 462 ± 13 g respectively (n = 21) (p = 0 8—0.7)

In the digitoxin group total haemoglobin was determined before and after medication in nine subjects, 2 men and 7 women. For the men the mean total haemoglobin was 800 g before and 845 g after digitalization. For the women the means were 514 g (range 423—600) before and 431 g (range 423—590) after

The haemoglobin concentration (g/l) was determined in all subjects before digitalization and in 13 after

Before digitalization the concentration was 13 2 ± 0 34 g/l S.D ±1.2 and 13 8 ± 0 26 0 9 g/l after (p = 0 1—0 05) The blood volume was in these cases 4.5 ± 0 25 l before and 4.3 ± 0 21 after There was no significant difference (p = 0.5—0 4)

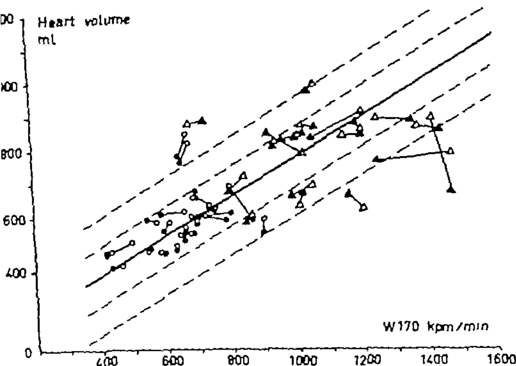


Fig. 5. Heart volume in relation to working capacity at pulse 170 W170.
Normal regression line and symbols as in fig. 3.

3. *Heart volume in prone position*

The heart volume in prone position was determined before digitalization in all subjects. After digitalization, prone heart volume determinations were for technical reasons made on 17 men and 18 women in the lanatoside C series and on 8 subjects (2 men and 6 women) in the digitoxin series.

The heart volume (in millilitres) was for men 814 ± 28 ml (S.D. ± 131) before lanatoside C and 807 ± 25 ml (S.D ± 102) after ($n = 17$). For women the figures were 566 ± 24 ml (S.D. ± 101) before and 557 ± 21 (S.D. ± 82) after ($n = 18$).

For the digitoxin group the figures for the 8 subjects were 669 (range 490—860) before and 642 (range 480—850) after.

There was no significant difference in prone heart volume either after lanatoside C or after digitoxin ($p = 0.2$—0.1).

4. *Heart volume in standing position*

It is well known that the heart volume is larger in the prone than in the standing position (Nylin 1934, Larsson & Kjellberg 1948, Kjellberg et al. 1949, 1951, Sjöstrand 1956, Linderholm & Strandell 1958). This has been explained on the basis of the redistribution of the blood volume which reduces the heart (Bjuhre & Laurell 1927, Jonsell 1939, Jon-

Table 7 Lanatoside C 1.6 mg i.v

Sex	Nr of subj	Work load kpm/min.	Heart rate beats/min.		Ventilation l/min. BTPS	
			before	after	before	after
M	16	rest	65 ± 2.4 10	61 ± 2.8 11	7.8 ± 0.40 1.4	8.2 ± 0.47 1.6
	11	300	100 ± 3.3 11	99 ± 3.6 12	20.3 ± 0.58 2.0	21.4 ± 0.84 2.8
	12	600	128 ± 3.5 14	1 8 ± 4.5 16	32.0 ± 0.68 2.4	33.9 ± 0.83 2.9
	11	900	158 ± 5.1 17	157 ± 4.3 14	45.7 ± 1.86 6.1	47.5 ± 1.78 6.2
	8	1200	168 ± 2.5 7	163 ± 3.5 9	60.7 ± 4.05 11.3	62.9 ± 5.04 14.1
F	16	rest	79 ± 3.4 13	69 ± 4.1 16	7.6 ± 0.51 2.1	7.4 ± 0.58 2.3
	16	200	106 ± 3.8 15	106 ± 1.4 6	18.6 ± 0.98 3.9	19.4 ± 1.4 5.7
	13	400	128 ± 6.1 22	126 ± 4.6 17	27.0 ± 1.19 4.3	27.1 ± 1.21 4.4
	11	600	155 ± 4.9 16	152 ± 4.4 15	39.3 ± 1.71 5.6	41.4 ± 2.7 8.9
	4	800	165 ± 3.5 7	160 ± 4.5 9	55.7 ± 3.25 6.5	52.6 ± 4.1 8.1

Table 8 Digitoxin

Sex	Nr of subject	Work load kpm/min.	Heart rate, beats/min.		Ventilation, l/min. BTPS	
			before	after	before	after
F	8	rest	69 ± 2.9 8	67 ± 3.9 11	7.4 ± 0.36 1.02	6.1 ± 0.35 0.7
	7	200	96 ± 3.6 9	95 ± 3.5 9	17.3 ± 0.79 2.1	15.7 ± 0.64 1.7
	8	400	120 ± 4.0 11	122 ± 4.9 14	26.1 ± 1.14 3.2	24.2 ± 1.23 3.5
	6	600	155 ± 3.2 8	153 ± 4.1 10	38.1 ± 3.5 8.6	35.6 ± 1.3 3.2

Table 7 (continued)

Oxygen uptake ml/min. NTPD		Mechanical efficiency per cent		Lactate, mM/l	
before	after	before	after	before	after
259 ± 10.5 40	293 ± 10.6 40			1.23 ± 0.12 0.44	1.30 ± 0.08 0.32
864 ± 24 79	887 ± 26.7 88	25.4 ± 0.39 1.3	23.4 ± 0.85 2.8	1.64 ± 0.22 0.74	1.54 ± 0.09 0.32
1418 ± 17.5 60	1445 ± 21.8 76	24.2 ± 0.27 0.9	24.6 ± 0.58 2.0	2.30 ± 0.30 1.10	2.34 ± 0.25 0.92
1980 ± 33.4 110	2047 ± 22.5 74	24.9 ± 0.34 1.1	25.4 ± 0.44 1.5	4.40 ± 0.57 1.88	4.39 ± 0.59 1.95
2603 ± 67.9 190	2620 ± 61.1 171	24.4 ± 0.57 1.6	24.4 ± 0.64 1.8	4.51 ± 0.64 1.8	5.06 ± 0.89 2.5
224 ± 2.5 58	212 ± 10.3 41			1.42 ± 0.11 0.42	1.50 ± 0.12 0.48
709 ± 23.2 100	690 ± 26.3 106	19.5 ± 0.88 3.4	19.5 ± 0.86 3.4	1.61 ± 0.13 0.51	1.84 ± 0.18 0.71
1001 ± 21.9 79	986 ± 22.6 82	24.6 ± 0.43 1.7	24.0 ± 0.65 2.5	2.25 ± 0.25 0.84	2.16 ± 0.21 0.76
1583 ± 28.2 93	1408 ± 38.9 129	24.7 ± 0.50 1.8	23.9 ± 0.66 2.2	3.72 ± 0.39 1.29	3.76 ± 0.32 1.09
1812 ± 53.2 106	1771 ± 92 183	24.0 ± 0.60 1.2	24.7 ± 0.85 1.9	5.14 ± 0.48 0.95	4.95 ± 0.44 0.87

Table 8 (continued)

Oxygen uptake ml/min NTPD		Mechanical efficiency per cent		Lactate, mM/l	
before	after	before	after	before	after
254 ± 14 40	218 ± 6 17			1.2 ± 0.08 0.25	1.4 ± 0.11 0.31
734 ± 35 93	667 ± 41 116	19.5 ± 1.01 2.7	20.4 ± 1.8 4.8	1.3 ± 0.06 0.16	1.5 ± 0.11 0.29
1021 ± 60 170	1025 ± 60 170	24.1 ± 1.2 2.9	24.2 ± 0.98 2.8	2.1 ± 0.35 1.0	2.1 ± 0.15 0.4
1356 ± 12 29	1391 ± 40 99	25.5 ± 0.63 1.4	24.7 ± 0.17 0.4	3.1 ± 0.41 1.0	4.0 ± 0.34 0.83

Table 9 Mechanical efficiency
Comparison between 1) use of measured resting value, 2) use of basal O_2 uptake according to Harris-Benedict.

Sex	Load	n		$\bar{x}$	$\bar{d}$	P
F	300	23	1 2	23 4 22 0	— 1 4	<0.001
	600	26	1 2	25.2 24.3	— 0.9	<0.001
	900	26	1 2	25.2 24.6	— 0.6	<0.001
	1200	12	1 2	25 4 24 9	— 0.5	<0.001
M	?00	41	1 2	20.2 19 1	— 1 1	<0 001
	400	41	1 2	24 9 23.8	— 1 1	<0 001
	600	34	1 2	24.8 23 7	— 1 1	<0.001
	800	8	1 2	24 7 24 0	— 0.7	< 0.001

sell & Sjöstrand 1941) The reduction of heart volume is accompanied by a change of pulse rate (Larsson & Kjellberg 1948) and stroke volume (Kjellberg et al 1951)

However there need not be any definite correlation between prone heart size and pulse rate (Linderholm & Strandell 1958) Since at the beginning of the lanatoside C series of experiments, it was found that the standing heart rate increased after digitalis glucoside, the heart volume both in standing and prone positions was determined in 23 subjects before and after intravenous administration of 1 6 mg of lanatoside C. Before administration it was 640 ± 32 ml (S.D ± 153) in the standing and 693 ± 32 ml in the prone position. The difference between standing and prone was highly significant (p <0.001)

After lanatoside C the standing heart volume was 607 ± 30 ml (S.D ± 144) and the prone 685 ± 32 Here again the difference between standing and prone was highly significant (p <0 001) Before digitalization the standing heart volume therefore was 640 ml, and after 607 ml. The difference in standing heart volume before and after lanatoside C was probably significant (p = 0 05—0 02) but this difference is on the other hand not striking when it is thought that the error of measurement was about 4 %

The conditions were similar in the digitoxin group. The standing heart volume was on an average 619 ± 49 ml (S.D ± 146) before and 557 ± 53 ml (S.D ±

150) after digitalization (n = 8) Again the difference was probably significant (p = 0.05—0.02)

Before digitalization there was in my material no correlation between standing heart volume and standing heart rate (r = —0.35 p = 0.5—0.1) After digitalization the correlation was probably significant (p = 0.05—0.02) though still weak (r = —0.42)

5 *Relationships between Different Circulatory Dimensions and W_{170}*

Mean values of W_{170}, prone heart volume and total haemoglobin have been reported in previous sections. Kjellberg et al (1949) showed that there is a linear relationship between W_{170} on the one hand and prone heart volume and total haemoglobin on the other

In figs 3 4 and 5 the individual values before and after digitalization are plotted in relation to the normal regression line.

6 *Effect on Oxygen Consumption Mechanical Efficiency and Lactate Concentration*

The values for heart rate, oxygen consumption, mechanical efficiency and lactate concentration are summarized in tables 7 and 8. From tables 7 and 8 it will be seen that V_{O_2} per work load is roughly at the same level as found by P O Astrand (195) and I Astrand (1960) for submaximal exercise. After digitalization no appreciable change occurs either with lanatoside C or digitoxin. The same applies to the lactate concentration.

On the other hand the values for mechanical efficiency were throughout higher in my material than those of P O Astrand (1952) and I. Astrand (1960) This is probably because I subtracted the measured resting value for V_{O_2} while P O and I Astrand used a value predicted as "basal" from tables. For the sake of comparison I have calculated the mechanical efficiency on a larger material in both ways, i.e. using either the measured resting value or a value predicted from Harris-Benedict's tables.

From table 9 it will be seen that there is a systematic difference between the two sets of values, the mechanical efficiency being constantly lower when predicted from tables. The difference is highly significant. It is then found that, using the same bases of calculation as I and P O Astrand, my figures of mechanical efficiency at least as far as women are concerned, are the same as found by I Astrand in 1960 For men my values are rather higher than reported by P O Astrand in 1952. There is no difference in mechanical efficiency before and after cardioactive glucosides. ($p > 0.1$) (fig 6)

7 *Effect on Blood Pressure*

There was no effect on either the systolic or diastolic blood pressure (measured by the cuff method) at rest or standing after injection of lanatoside C. The systolic blood pressure before digitalization was 121±2.5 (S.D 12, n = 22) and the diastolic 78±2.0 (S.D 5 2) The corresponding values after digitalization were 120±2.8 (S.D 12.4) and 76±2.2 (S.D 10) In standing position the systolic blood pressure before digitalization was 119 ± 3.7 and the diastolic 79 ± 2.1 After digitalization the corresponding values were 117 ± 2.6 and 73 ± 2. The differences in blood pressures before and after digitalization are not significant (p = 0.5—0.2)

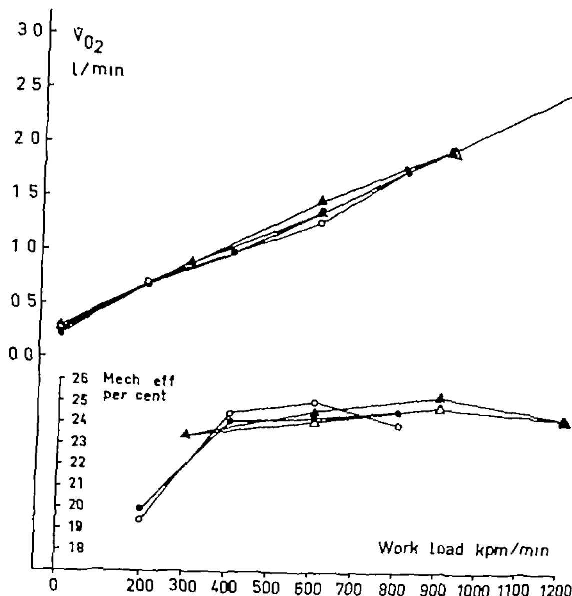


Fig 6. Oxygen uptake and mechanical efficiency in relation to work load before and after digitalization. (Open symbols = before and filled symbols after)

Discussion

Subjective symptoms

Are subjective symptoms of any kind a *sign of toxicity and by definition thus* also of full digitalization or are e.g. gastrointestinal symptoms after oral doses a sign of local irritation and therefore no definite evidence of digitalization?

The galenicals are generally considered to have an irritative effect on the gastrointestinal tract. Gold (1942) compared single doses of folium digitalis with, as he considered, equivalent doses of digitoxin and found a considerably higher percentage of subjective symptoms in the folium group. If nausea occurs after parenteral *administration, it is ascribed to systemic* toxicity. Opinions are divided, however concerning oral medication. Gold et al. (1952) after clinical experiments in man drew the conclusion that vomiting after

oral administration of certain cardioactive glucosides is due chiefly or perhaps solely to a local gastrointestinal irritation since after oral administration of digitalis, vomiting does not necessarily coincide with other signs of toxicity and, above all, because the quantities presumed to be absorbed were sub-emetic on parenteral administration. The doses used by Gold et al. varied fairly considerably and can hardly be called equivalent. Moreover the experiments were made on cardiac patients in different degrees of incompensation. Earlier Gold et al. (1950) had shown that there is a latent period of 8—10 hours fter the last dose of digitalis before the onset of the emetic effect. The latent period for digitoxin was stated to be 6 ½—21 hours and for digoxin 1—3 hours (Gold et al. 1952).

Hatcher et al. (1922) reported a central ffect of digitalis. The same conclusion was reached by Borison & Wang (1951) and Borison (1952) who, in experiments on cats, showed that digitalis has both a central effect localized to the postramus area of the medulla, which they called the emetic trigger zone, and a peripheral ffect which is not gastrointestinal but whose exact nature it has not yet been possible to determine. According to these thors the intestine plays ery small rol or none t all, in digitalis vomiting. They speak both of early omiting, which follows on an intravenous injection and is alw ys arranged by a trigger zone ope ration, being thus of central origin, and of late vomiting which is neither of central nor gastrointestinal origin.

I Gold' experiments the period of latency was relatively long before symptoms ppeared after oral administration of digitoxin. As appears from table 1 B, the period of latency after oral digitoxin in my experiments was also fairly long, between 5 and 12 hours, except in one case when it was 3 hours after the last dose. So long a latent period does not tally with the symptoms being due solely to a local gastrointestinal effect.

But the situation appears to be different as regards the subjects who had nausea after a large single dose of digoxin. In these cases the latent period for nausea was short, 40 minutes to " hours generally 1 hour (table 1 C) and the effect rapidly disappeared. Here the effect may be solely local. Gold et al. (1952) found roughly the same latent period for digoxin. Nausea after intravenous lanatoside C cannot rea sonably be of gastrointestinal origin.

As regards the present investigations, it may be said in conclusion that subjective symptoms after digitoxin and lanatoside C may be regarded as at least partly a general effect and by definition as a sign of full digitalization whereas this probably should not apply to the digoxin group since their subjective symptoms followed so quickly after digitalization. The degree and nature of the symptoms were in most cases relatively mild, which conforms with earlier reports in the literature of the effect of digitalis in healthy persons. The symptoms are not so grave or lengthy as reported in clinical materials, although the doses used in the present study were of the same order of magnitude as in current clinical use. It is probable, therefore, that the origin and severity of the symptoms are dependent to a great extent on the clinical status of the patients.

Heart rate at est

Usually the resting heart rate falls to a varying extent after digitalization of healthy subjects (Routier & Puddu 1935 Larsen & Nielsen 1936, Nielsen et al. 1939 Erickson & Fahr 1945 Koelbing 1950 Yu et al. 1952, Seltzer et al. 1959). The effect disappears fairly quickly (e.g.

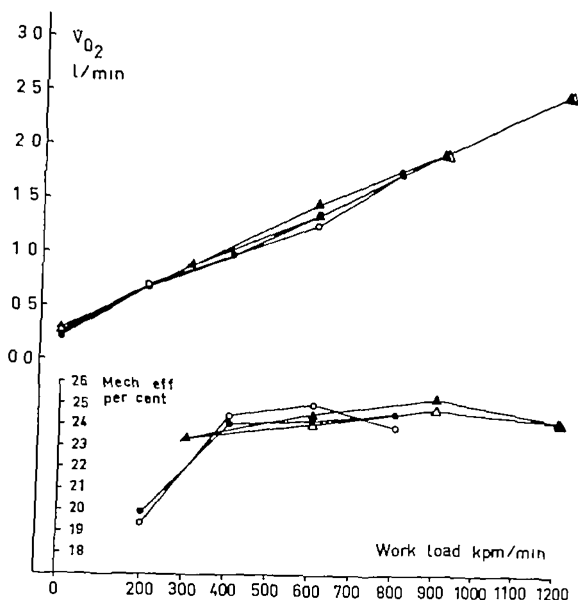


Fig 6. Oxygen uptake and mechanical efficiency in relation to work load before and after digitalization. (Open symbols = before and filled symbols after)

Discussion

Subjective symptoms

Are subjective symptoms of any kind a sign of toxicity and by definition thus also of full digitalization or are e.g. gastrointestinal symptoms after oral doses a sign of local irritation and therefore no definite evidence of digitalization?

The galenicals are generally considered to have an irritative effect on the gastrointestinal tract. Gold (1942) compared single doses of folium digitalis with, as he considered, equivalent doses of digitoxin and found a considerably higher percentage of subjective symptoms in the folium group. If nausea occurs after parenteral administration it is ascribed to systemic toxicity. Opinions are divided, however concerning oral medication. Gold et al. (1952) after clinical experiments in man, drew the conclusion that vomiting after

creased highly significantly. This orthostatic reaction is still more pronounced in the subjects in the lanatoside C group who had subjective symptoms. The remarkable thing is that the digitoxin group did not react in this way: the standing heart rate was not changed by digitoxin, nor the orthostatic pulse reaction either. This is especially evident from table 3, which shows the results for those who received both lanatoside C and digitoxin. Nor did this increased orthostatic pulse reaction occur after digoxin. In respect of the orthostatic pulse reaction the lanatoside C, digitoxin and digoxin groups did not differ from one another prior to digitalization. The subjects represent a normal material, and the standing heart rate and orthostatic pulse reaction were comparable with the findings in other normal materials composed of individuals with varying degree of training (Holmgren et al. 1957).

It is possible that the difference in orthostatic reaction after lanatoside C, digitoxin and digoxin is caused by differences in the conditions of the tests in the present study.

Lanatoside C was injected. This may have created anxiety in the subjects, with consequent cardio-acceleration. There was, however, no difference in heart rate between the various series. Nor did the O_2 uptake at rest differ before and after lanatoside C. And, furthermore, the orthostatic test was not made until 2—3 hours after the lanatoside C injection and the subjects were accustomed to the experimental routine.

Another explanation of the orthostatic reaction induced by lanatoside C is to be sought in a central nervous effect of the drug, the effect of vagal tone may be accompanied by a decreased sympathetic tone, e. g. to peripheral resistance and capacity of vessels. Through a compensatory mechanism this would give rise in the standing position to a high sympathetic tone with a marked increase of the heart rate in consequence. An effect on the peripheral mechanisms of vascular control cannot be ruled out, however.

The reaction in my experiments was greatest in the lanatoside C group with subjective symptoms, that is in the subjects in whom the digitalis effect was most definitely documented, and may be explained as above. I have observed a similar orthostatic reaction in a patient who had taken 15 g folium digitalis tablets for suicidal purposes. She was gravely intoxicated and had A-V block II with a heart rate of 35 beats/min. Her standing heart rate, on the other hand, was 95 beats/min. without A-V-block. It is difficult, however, to explain the difference in reaction between the lanatoside C group and the digitoxin group.

Effect on Circulatory Adjustment during Exercise

During work of low intensity the heart rate after digitalization was slightly lower than before. This is in agreement with the findings of Yu et al. (1952) and Williams et al. (1958) who used low loads throughout. At a higher working intensity, on the other hand, there was no significant difference in heart rate, ventilation, oxygen uptake or lactate concentration during exercise with or without digitalis. Thus digitalis did not affect the calculated physical working capacity at maximal heart rate nor the working capacity at heart rate 170.

In general it may be said that — apart from the effect at low loads — digitalis does not change the circulatory response to exercise as regards the magnitude of the stroke volume, the increase of heart rate and cardiac output, the adjustment of the peripheral circulation or the mechanical efficiency.

Seltzer et al 1959 Koelbing 1950) The degree of lowering in relation to the premedication situation is not particularly great, however Yu et al (1952) found the reduction to be significant at the 1 per cent level. Seltzer et al. (1959) found that the heart rate admittedly fell considerably in individual cases but that the mean change in the group was not significant. The circumstances were similar in the present study A few subjects had a greatly reduced heart rate, others a somewhat higher rate, and in some it was unchanged The mean change for the entire material was comparatively insignificant. In this respect a difference appears to exist between those who had subjective symptoms after digitalis (both lanatoside C and digitoxin) and those who remained free from symptoms. In the former group the reduction of heart rate after digitalization was probably significant In those without symptoms there was no significant difference. It is possible that if all subjects had been in the same and fairly pronounced state of nausea, i.e. of fairly pronounced intoxication the effect might have been more uniform. But in experiments of this type it is hardly defensible to drive the degree of digitalization too far in healthy persons.

Vagal effect

The digitalis glucosides have been said to have a potentiating influence on the effect of vagal activity to which is ascribed the reduced heart rate (Eichna et al. 1943 and others) Heymans & Heymans (1926) showed in cross-circulation experiments that digitalis does not have a direct effect on the vagal centres. They considered the primary bradycardia after injection of digitalis to derive from a reflex mechanism. Gremels (1935) considered that small quantities of strophanthin provoked an increased irritability of the vagus nerve, while Abdon & Nielsen (1938) thought that the cardioinhibitory effect of digitalis on the vagal nerve was probably due solely to sensitization of the heart muscle to normal vagal tone. Later on, the question of the vagal effect of digitalis was reformulated by Abdon et al (1938) who maintained that effect on the myocardium was instead due to sensitization to acetylcholine

Gold et al. (1939) after experiments on patients with atrial fibrillation before and after digitalis, thought the slower heart rate to be due to a combination of two factors a vagal factor which is abolished by atropine and exercise, and an extravagal factor which is unaffected by atropine, and that the vagal factor generally predominates after moderate doses, the extravagal after large doses.

Discussion of the vagal effect has now fallen into the background. There is no doubt that digitalis does have a vagal effect, but this cannot be isolated from its relation to the haemodynamics as a whole and in itself probably plays a comparatively minor role.

The orthostatic pulse reaction

The orthostatic pulse reaction, that is the difference between resting and standing heart rate, increased greatly after intravenous injection of lanatoside C, both after small doses and after 1 6 mg This increased pulse reaction might conceivably be due to the lower resting pulse rate and therefore be merely an apparent increase As already noted the resting heart rate was admittedly rather lower but not significantly so on the other hand the standing heart rate was higher after lanatoside C both in small and large doses, the rise being probably significant ($p = 0.02$—0.01) The orthostatic pulse reaction in

Electrocardiographic Changes in conjunction with Digitalization

Methods of measurement

P-Q and Q-T

The measurements of the duration of P-Q (or P-R) and Q-T (or R-T) were made within the same R-R interval as was used for determination of the duration of R-R. The unit of measurement was 0.01 second and the durations are indicated as means of at least three measurements. In most cases P-Q and Q-T were measured in lead II and CR2 or CH2. CR2/CH2 were chosen since ST-T changes due to digitalization proved to be least pronounced in these leads.

The *Q-T measurements* have been difficult to perform, especially on electrocardiograms taken during exercise at high frequencies on which it was often impossible to decide where the T wave ends, an experience which is shared by all who have had to do with this problem (*inter alia* Grewin 1948, Lepeschkin & Surawicz 1952, Carlson & Sjöstrand 1955, Bengtsson 1956) the percentage of rejects has consequently been fairly high.

Q-T is not indicated in absolute figures but has been corrected for the frequency and is reported as an index "I" taken from nomogram presented by Goldberger (1955).

The *ventricular gradient G* and the mean QRS axis were measured by the method described by Ashman (1943) Ashman, Gardberg & Byer (1943) Ashman & Byer (1943) and Gardberg (1957). The direction is indicated in degrees, the size in units (one unit is equal to 4 microvolt seconds)

ST-T

The isoelectric line has been drawn between two P-Q junctions and the ST segment has been evaluated in relation to this isoelectric line. The ST segment was measured at the junction and depressions have been measured in units of 0.5 mm (1 mm = 0.1 mV)

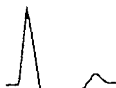
The T waves were measured in units of 0.5 mm from the isoelectric line and their heights at rest and after exercise were statistically analysed. The T wave was also evaluated in conjunction with the ST segment, and the negative phase of the T wave has been calculated from the isoelectric line. For ST-T all leads were separately evaluated.

The importance of not only measuring ST-T depressions in absolute terms but also of describing the appearance and course of the ST-T segment has often been emphasized (Bengtsson 1957 Sandberg 1961 Lloyd-Thomas 1961). When ST is measured and reported in absolute terms, as done, for example, by Grewin (1948) and Bengtsson (1956, 1957) the results can be statistically analysed, which is necessary for establishing normal variations. One loses the description of form, however and at the same time the overall evaluation of the ST-T region. All types of ST-T changes observed have earlier been described on the bases of measurements and analysis of the ST-T changes that appear in healthy subjects after digitalization (Nordström—Öhrberg 1959). This description has been used in the pre

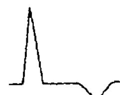
It also appears from these investigations that the degree of orthostatism in the standing position does not influence the circulatory response during sitting exercise. This is corroborated by the fact that the increase of heart rate during exercise after lanatoside C did not differ significantly from that after other digitalis drugs which produced no marked orthostatic reaction.

The lactate concentrations during exercise showed large intra- and interindividual variations, as earlier found in untrained individuals (Holmgren & Ström 1959, I. Åstrand 1960). The relatively *high lactate concentrations* showed that exercise at the highest loads introduced a fairly large anaerobic component into the total muscular metabolism, but that the exercise was not maximal. The lactate concentration values at the end of exercise were within the same order of magnitude before and during digitalization, indicating that the medicamentation did not influence the ratio between aerobic and anaerobic metabolic components during exercise.

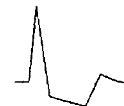
In conclusion, these studies have shown that the the digitalis glucosides do not provoke such a haemodynamic effect on healthy subjects as to influence the circulatory response to an exercise test at submaximal loads to any important degree.



ST segment horizontally depressed >0.1 mV — ≧0.2 mV
T wave diphasic, the negative component = 0.2 mV



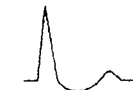
T wave negative >0.1 mV ≦0.4 mV



ST segment obliquely downward sloping >0.2 mV ≦0.4 mV
T wave diphasic, the negative component ≧0.4 mV



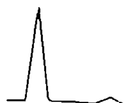
ST segment horizontally depressed 0.3—0.4 mV T w e dipha sic, the negative component 0.3—0.4 mV



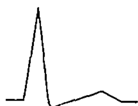
ST segment concave, sagging, what is called in German mul-denförmig" Inconsiderable or no depression at junction, the depression at its maximum in the middle 0.1—0.2 mV

TYPES OF ST T CHANGES

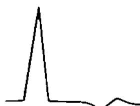
(Fig 7)



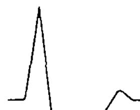
T wave ≤0 1 mV



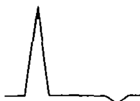
ST depressed = 0 1 mV with normal configuration, T wave positive ≧0 1 mV



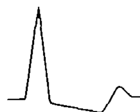
T wave diphasic (the negative component ≤ 0 1 mV)



ST segment horizontally depressed ≤ 0 1 mV T wave diphasic.



T wave negative ≤ 0 1 mV



ST segment depressed 0 1 mV at junction, obliquely downward sloping, T wave diphasic, the negative component of the T wave > 0 1 mV but < 0.3 mV

beats/min after digitalization her heart rate was on an average 72 beats/min. She had also relatively pronounced subjective symptoms.

C. Digoxin

The P-Q interval for the entire material prior to digoxin was on an average 14.0 csec, after digoxin 15.1 csec (table 11). The difference was not significant (p = 0.1—0.05). No A-V block occurred.

2. Standing

A. Lanatoside C

Before digitalization with 1.6 mg lanatoside C the P-Q interval in standing position was 14.7 csec for men and 13.8 csec for women. After digitalization the figures were 15.1 and 14.8 csec. The P-Q interval was thus, on an average, rather longer while, as previously noted, the heart rate was higher. The increase of the P-Q interval was slight, however and not significant (p = 0.1—0.05). No subject had A-V block in standing position. The subject (no. 42) with prolonged P-Q interval at rest had a standing P-Q interval of 19 csec.

In the 0.4—0.8 mg groups the P-Q intervals before digitalization were 15.1± 2.4 csec, after 0.4 mg 15.5±2.1 csec and after 0.8 mg 15.5±2.1, in fact entirely similar values.

B. Digitoxin

The P-Q interval before digitoxin (table 10) was on an average 14.2 csec, and 15.8 csec after. The increase is not significant (p = 0.1—0.05).

One subject (no. 47) who at rest after digitoxin had A-V block II with P-Q interval up to 46 csec had a standing P-Q interval of 35 csec with heart rate of 96 beats/min.

C. Digoxin

The standing P-Q interval before digoxin (table 11) was 13.8 csec and 13.6 csec after (p = 0.7—0.6).

3. During exercise

A. Lanatoside C

During exercise at 900 kpm/min the P-Q interval for men was the same before and after digitalization (12.6 csec) and for women at 600 kpm/min 11.8 csec before and 12.5 csec after. The difference is not significant (p = 0.2—0.1).

B. Digitoxin

The P-Q interval was 11.6 csec before digitoxin and 11.7 csec after (table 10). Even the subject (no. 47) who at rest after digitalization had a P-Q interval of 46 csec had exactly the same P-Q interval during exercise before and after digitalization.

C. Digoxin

The P-Q interval did not change either after digoxin, as seen from table 11.

4. After exercise

A. Lanatoside C

Immediately after exercise the mean predigitalization P-Q interval for men was 14.4 csec, and after 1.6 mg lanatoside C 15.1 csec. The difference, however, is only probably significant (p = 0.05—0.02). The heart rate was, however, lower in the measured R-R interval after digitalization (table 9). For women, on the other hand, the P-Q interval after lanatoside C was 15.9 csec against 14.0 csec before, which is a significant difference (p = 0.01—0.001). The heart rate, however, is rather higher in the measured R-R interval after digitalization. One subject (no. 21) had a P-Q interval up to 26 csec after digitali-

sent study with minor modifications (fig 7)

Effect of cardioactive glucosides on rhythm and conduction

The results in respect of heart rate have been reported in an earlier section

Effect on the rhythm

At rest sinus arrhythmia and magnification of earlier sinus arrhythmia were common *In table 12 is shown the total incidence* of sinus arrhythmia arising after digitalization (about 30 *per cent of the* cases) which percentually was fairly evenly divided among the various glucoside groups. *In standing position* naturally enough, the incidence was very much lower occurring in only 7 digitalized subjects.

During exercise the arrhythmia disappeared entirely except in one subject at the lightest load. *Immediately after exercise* it reappeared but diminished again 5 minutes after exercise, being then recorded in only 9 cases.

Sino-atrial block was recorded in one *subject immediately after exercise*

The frequency of *premature beats* (atrial and ventricular premature beats) was extremely low One subject had ventricular premature beats resting standing, and during and after exercise, even before digitalization. In this person digitalization *produced no increase in the number of* premature beats. In other subjects occasional ventricular premature beats were recorded before digitalization.

After digitalization only occasional premature beats were recorded at rest, during and after exercise. At heavy loads no premature beats were recorded at all (table 12)

A V conduction

1 *At rest*

A Lanatoside C

Intravenous injection of 1 6 mg of lanatoside C produced a brief prolongation of the P-Q interval at rest both in men and women (table 9) *from 15 3 to 15 9 csec* for men and from 14 7 to 15 4 csec in women. The prolongation was thus slight, yet probably significant ($p = 0\ 05—0\ 02$)

In one case (no. 42) a conduction time of 19 csec at a heart rate of 76 beats/min was recorded before digitalization and 23 *csec at 95 beats/min after lanatosid C.*

In lower doses (0 4—0 8 mg) on the other hand lanatoside C produced no significant change in conduction time. Thus the P-Q interval before digitalization was on an average 16.3 csec ($n = 9$) after 0 4 mg 16.8 and after 0.8 mg 18.3 *($p = 0\ 1—0\ 05$) The greater prolongation* of the P-Q interval after 0.8 mg lanatoside C is due to a very considerable prolongation in a 17 year-old subject (no. 63) The predigitalization conduction time of this person was 21 csec at 68 beats/min, and after 0 8 mg his heart rate was 72 *beats/min and P Q interval* 35 csec. The P-Q interval of this subject was unaffected by 0 4 mg lanatoside C.

B *Digitoxin*

The atrioventricular conduction was generally little affected by digitoxin. Before administration the P-Q interval (table 10) was 14 8 csec, and 16.6 csec after The difference is not significant ($p = 0\ 2 — 0\ 1$) *One subject (no. 47)* had a greatly prolonged P-Q interval (up to 46 csec) with dropped ventricular *complex*, A V block II (7 6 6 5 5 4 block with Wenckebach periods) This subject *(age* 48 years) had a P-Q interval before digitalization of 19 csec at a heart rate of 60

P-Q, msec			I"		
before	after		before	after	
15.3 ± 0.3 1.6	15.9 ± 0.4 1.9	= 21	0.93 ± 0.012 0.06	0.90 ± 0.014 0.06	n = 21
14.7 ± 0.4 1.8	15.1 ± 0.4 1.9	= 20	0.95 ± 0.015 0.07	0.96 ± 0.017 0.08	n = 21
12.6 ± 0.3 1.3	12.6 ± 0.4 1.5	= 15	0.98 ± 0.010 0.04	0.97 ± 0.009 0.02	= 16
14.4 ± 0.4 1.5	15.1 ± 0.5 2.0	= 19	0.96 ± 0.014 0.06	0.95 ± 0.021 0.09	n = 19
15.6 ± 0.4 1.8	17.0 ± 0.8 3.6	= 21	1.00 ± 0.015 0.07	0.99 ± 0.012 0.06	= 21
14.7 ± 0.5 2.4	15.4 ± 0.7 2.9	= 20	0.98 ± 0.017 0.07	0.92 ± 0.018 0.08	n = 20
13.8 ± 0.5 2.1	14.8 ± 0.6 2.7	19	0.95 ± 0.05 0.2	0.98 ± 0.02 0.07	= 19
11.8 ± 0.4 1.3	12.3 ± 0.4 1.3	13	0.99 ± 0.016 0.06	0.99 ± 0.016 0.06	= 12
14.0 ± 0.5 1.96	15.9 ± 0.7 2.8	= 15	0.95 ± 0.014 0.05	0.94 ± 0.03 0.12	= 12
15.0 ± 0.5 1	16.2 ± 0.8 3.6	= 20	1.03 ± 0.013 0.06	1.02 ± 0.011 0.05	= 20

Table 9
P Q and R R intervals n csec (1/100 sec) and Q T interval expressed as index "I" before and after ad ministration of 1 6 mg Lanatosid C intravenously
(M ± S.E., S.D)

Sex		R R, csec	
		before	after
Males	resting	95 6 ± 3 7 16.8 n = 21	99 9 ± 4 1 18.7
	standing	81 7 ± 2.6 11 7 n = 21	74 4 ± 3 2 14.5
	work 900 kpm/min.	37.8 ± 2.5 10 4 n = 18	39 4 ± 1.0 4.2
	immediately after work	55 7 ± 1.5 6.6 n = 21	57 4 ± 2 7 12.0
	3 min. after wo k	64 4 ± 1 4 6.6 n = 21	6[illegible].5 ± 1 4 6.3
Females	resting	83 7 ± 2.8 12 7 n = 20	93 0 ± 5.2 23.2
	standing	76.4 ± 4 4 14.8 n = 19	68.5 ± 4.3 19 1
	work 600 kpm/min.	37 7 ± 0.9 3 7 n = 16	37.9 ± 1 0 4 0
	immediately after work	57.5 ± 3 2 12.2 n = 14	56 4 ± 3.1 11 6
	3 min. after work	64.3 ± 2.2 10.0 n = 20	63.[illegible] ± 2.5 11 2

P-Q, msec		"T"	
before	after	before	after
14.8 ± 0.5 2.3 (= 23)	16.6 ± 1.6 7.0 (= 23)	0.94 ± 0.014 0.065 (= 23)	0.86 ± 0.013 0.060 (= 19)
14.2 ± 0.5 2 (= 22)	15.8 ± 1.2 5.3 (= 20)	0.95 ± 0.015 0.072 (= 23)	0.91 ± 0.012 0.052 (= 19)
11.6 ± 0.3 1.1 18	11.7 ± 0.3 1.2 (18)	0.95 ± 0.019 0.067 (12)	0.93 ± 0.012 0.039 (= 10)
14.6 ± 0.58 2.61 20	16.1 ± 1.54 6.73 = 19)	0.95 ± 0.016 0.069 (= 19)	0.91 ± 0.012 0.049 (n = 18)
15.1 ± 0.48 2.9 = 23	17.1 ± 1.40 6.12 19)	0.99 ± 0.015 0.073 (= 23)	0.94 ± 0.014 0.059 = 19)

P-Q, msec		"T"	
before	after	before	after
14.0 ± 0.6 1.9 10	15.1 ± 0.5 1.7 10)	0.90 ± 0.02 0.07 (= 10)	0.89 ± 0.02 0.07 (= 10)
13.8 ± 0.4 1.2 10	13.6 ± 0.6 2.0 10)	0.95 ± 0.02 0.05 = 10)	0.94 ± 0.01 0.04 (10)
12.2 ± 0.3 0.8 6	11.7 ± 0.2 0.5 6)	0.97 ± 0.03 0.07 = 6)	0.97 ± 0.03 0.08 = 6
14 0.3 1.0 10	13.9 ± 0.5 1.6 10	0.91 ± 0.01 0.04 (10	0.92 ± 0.02 0.05 = 10)
14.8 0.6 1.8 10	15.1 ± 0.5 1.6 10	0.98 ± 0.02 0.05 10)	0.96 ± 0.02 0.06 (= 10)

Table 10
P-Q and R R intervals in csec (1/100 sec) and Q T interval xpr ssed as index I" befo e nd after ad ministration of digitoxin
(M ± S E, S.D)

	R R, csec	
	before	after
resting	91.5 ± 3 0 14 4 (n = 23)	99.5 ± 3.3 14 7 (n = 23)
standing	77.9 ± 3 2 15 3 (n = 23)	73 0 ± 2.9 13 0 (n = 23)
during work	36.6 ± 0 7 2.9 (n = 16)	37 1 ± 0 7 2.4 (n = 16)
immediately after work	55 6 ± 1.9 8.7 (n = 20)	61 4 ± 3 1 13.9 (n = 20)
3 min. after work	64.5 ± 1.57 7.55 (= 23)	68 2 ± 2.57 11.21 (n = 23)

Table 11
P-Q and R R intervals in csec (1/100 sec) d Q T interval expressed as dex I befor nd fter ad
ministration of digoxin
(M ± S E, S.D)

	R R, csec	
	before	after
resting	98.4 ± 4.8 15.3 (n = 10)	100.5 ± 6 1 19.3 (= 10)
standing	83 7 ± 5 5 17.5 (n = 10)	77 5 ±5 1 16.1 (= 10)
during work	40 3 ± 1.3 3 1 (n = 6)	39 2 ± 0 9 2.3 (n = 6
immediately after work	60 9 ± 4 1 13 1 (n = 10)	60 4 ± 3 8 12.2 (= 10)
3 min after work	68 3 ± 3 0 9 4 (= 10)	71 0 ± 3 4 10.8 (= 10)

P-Q caec		I	
before	afte	before	after
14 8 ± 0.5 2.3 (= 23)	16.6 ± 1.6 7.0 (= 23)	0.94 ± 0 014 0 063 (= 23)	0 86 ± 0 013 0 060 (= 19)
14 2 ± 0.5 2.2 (= 22)	15.8 ± 1.2 5 3 (= 20)	0 95 ± 0 015 0.072 (= 23)	0.91 ± 0 012 0.052 (= 19)
11 6 ± 0 3 1 1 = 10)	11 7 ± 0.3 1 2 (= 18)	0 95 ± 0 019 0 067 (= 12)	0 95 ± 0.012 0.039 (n = 10)
14 6 ± 0.58 2 61 = 20)	16 1 ± 1 54 6.73 (= 19)	0.95 ± 0 016 0.069 (= 19)	0 91 ± 0 012 0 049 (= 18)
15 1 ± 0 48 29 = 23)	17 1 ± 1 40 6.12 (= 19)	0 99 ± 0 015 0 073 (= 23)	0.94 ± 0 014 0 059 (= 19)

P-Q caec		"T"	
before	after	before	after
14 0 ± 0 6 1 9 10	15 1 ± 0 5 1 7 (= 10)	0.90 ± 0.02 0 07 (= 10)	0.89 ± 0.02 0.07 (= 10)
15 8 ± 0 4 1 2 10	15 6 ± 0 6 2.0 (= 10	0 95 ± 0 02 0 05 = 10)	0.94 ± 0 01 0.04 (= 10)
12 2 ± 0 3 0 8 6	11 7 ± 0.2 0.5 (= 6)	0.97 ± 0.03 0 07 (= 6)	0.97 ± 0 03 0.08 (= 6)
14 2 ± 0 3 1 0 10	13 9 ± 0 5 1 6 (10)	0 91 ± 0.01 0 04 (= 10)	0.92 ± 0 02 0.05 (n = 10)
14 8 ± 0 6 1 8 10	15.1 ± 0 5 1.6 = 10)	0 93 ± 0 02 0 05 (= 10)	0 96 ± 0 02 0 06 (= 10)

Table 12 Changes in rhythm and conduction following 92 digitalizations

	at rest	standing	exercise	After exercise		Total
				Imm. after	3 min. after	
sinus arrhythmia	30	7	0	18	9	64
sino-atrial block	0	0	0	1	0	1
auricular premature beats	2	0	0	1	0	3
ventricular " "	1	1	0	?	0	4
A V block I and II	2	1	0	3	3	9
Total	35	9	0	25	12	81

zation. In this subject the conduction time varied as 17—24—26 csec before digitalization her P Q interval was 18 csec. Her heart rate on the other hand was fairly similar before and after digitalization (127 beats/min before and 123 after) No other subject had a P-Q interval of such magnitude that one can speak of a block.

With lanatoside C in smaller doses (0.8—0 4 mg) there was no significant change in P-Q interval.

Three minutes after exercise the P-Q intervals both for men and women were still rather longer after the largest dose of lanatoside C. Before digitalization the men had a P-Q interval of 15 6 csec, and after 17 0 csec, the difference being probably significant (p = 0 02—0 01) For women the corresponding figures were 15 0 and 16.2 csec (p = 0 02—0.01) One digitalized subject (no. 52) three minutes after exercise had a P-Q interval of 30 csec before digitalization her figure was 18 csec. Her heart rate before and after digitalization was, respectively 90 and 94 beats/min. Subject no. 21 who had a P-Q interval of 26 csec immediately after exercise had the same interval three minutes after exercise.

Nor with the lower doses of lanatoside C was there any significant change of the P-Q interval three minutes after exercise

B. *Digitoxin*

Immediately after exercise the P-Q interval in this group was 14 6 csec before digitalization, 16 1 csec after (table 10) The difference is not significant (p = 0 2—0 1) Two subjects after digitalization had a considerably prolonged P-Q interval One subject (no 44) had before digitalization a P-Q interval of 17 csec and heart rate of 113 beats/min after digitalization the P-Q interval immediately after exercise was 24 csec and the heart rate 120

The second subject (no 47) immediately after exercise had a P-Q interval of 19 csec before digitoxin and after digitoxin 42 csec, A-V block II with Wenkebach periods, which she also had at rest after digitalization.

Three minutes after exercise the P-Q interval was 15 1 csec before digitoxin and 17 1 after (S.D ±6.12) the difference, despite the large increase, being non-significant (p = 0 2—0.1)

One subject (no 44) who immediately after exercise had a P-Q interval of 24

csec, now had a P-Q interval of 17 csec at heart rate 116 against 16 csec at heart rate 95 before digitalization.

C. Digoxin

From table 11 it will be seen that the P-Q interval before digoxin was 14.2 csec immediately after exercise and 14.8 csec three minutes after exercise. The corresponding figures after digoxin were 13.9 and 15.1 csec. The differences were not significant (p = 0.5—0.4 and 0.6—0.5 respectively)

Q-T interval

A. Lanatoside C

The Q-T interval indicated as an index" will be seen in tables 9, 10 and 11. *At rest* the predigitalization figures were 0.93 for men and 0.98 for women. After 1.6 mg of lanatoside C they were 0.90 and 0.92. The difference is significant for women (p = 0.01—0.001) and not significant for the male subjects (p = 0.2—0.1)

In standing position and during and after exercise the index did not differ significantly (p = 0.1—0.05) either in women or men (table 9)

After smaller doses of lanatoside C (0.4—0.8 mg) the index at rest was 1.00 ± 0.06 before and 0.99 ± 0.04 after 0.4 mg, and 1.0 ± 0.09 after 0.8 mg. The differences are not significant (p>0.1). In standing position the index was 1.0 ± 0.05 before and, respectively 1.0 ± 0.05 and 1.0 ± 0.03 after 0.4 and 0.8 mg lanatoside C (n = 9) i.e. no difference.

During exercise the index was 0.9.. ± 0.07 before and, respectively 0.97 ± 0.05 and 0.98 ± 0.03 after 0.4 and 0.8 mg. Here again there was no significant difference. Immediately after exercise the index was 0.97 ± 0.04 before and, respectively 0.95 ± 0.05 and 0.94 ± 0.03 after 0.4 and 0.8 mg (n = 9, p >0.1). Three minutes after exercise the indices were identical before, after 0.4 and after 0.8 mg lanatoside C, namely 1.0 ± 0.04, 1.0 ± 0.05 and 1.0 ± 0.04

Digitoxin

At rest the index was 0.94 before and 0.86 after digitoxin. The difference was highly significant (p<0.001). In standing position the figures were 0.95 before and 0.91 after digitoxin. This difference was not significant (p = 0.1—0.05)

The index did not differ either during exercise. Immediately after exercise the figures were 0.95 before and 0.91 after digitoxin (p = 0.2—0.1) and three minutes after exercise 0.99 and 0.94: the difference is significant (*p = 0.01—0.001*) (table 10)

Digoxin

As will be seen from table 11 the indices are practically identical before and after oral digoxin at rest, standing, and during and after exercise.

The effect of cardioactive glucosides on the ST-T region

1. ST-T reaction before digitalization

As appears from table I, the majority of cases exhibit an isoelectric or slightly elevated ST region and positive T wave >0.1 mV at rest, standing, and in conjunction with exercise. A small number were grouped as 1—2 in standing, during and after exercise (For explanation of symbols see fig 7). Four other cases had T wave changes of type 4—5 in standing position. In conformity with earlier opinions (Michel 1954, Bengtsson 1956 and Sandberg 1961) this has been regarded as a non-pathological reaction.

I *ECG before digitalization (n=62)*

Type of change	Resting	Standing	During exercise	After exercise	
				immed.	3 min.
0	61	50	55	58	55
1—2	1	8	7	4	7
4		2			
5		2			

2 *ST T changes induced by digitalis*

A. *Lanatosid C 1.6 mg*

The ST T changes arising after injection of 1 6 mg lanatoside C are shown in tables 13 14 15 16 and 17

At rest

The height of the T waves in the various leads before and after administration of lanatosid C will be seen from table 13 The T waves were tahroughout *lower after digitalization, the difference* before and after digitalization being highly significant ($p < 0.001$)

The ST T changes and their distribution in different leads after the digitalization are shown in table 14 Most electrocardiograms were unchanged and when *changes were recorded they were generally* mild.

Thus table shows the number of subjects who revealed changes in any lead after digitalization. They are divided into groups as follows and as shown in fig 7 Group O no changes groups 1—2 changes of types 1—2 groups 3—10 changes of *types 3—10* and *group 11* It will be seen that 64 % of the subjects had unchanged ST T regions, 14 % mild changes of types 1—2, 20 % changes of types 3—10 and only 2 % changes of type 11 (table II)

Standing

After 8 minutes standing, changes due *to digitalization were recorded both in a* greater number of subjects and of more types as at rest. The results are shown in *table 15*

II *ST T changes at rest*

Group	No. of subjects	%
0	27	64
1—	6	14
3—10	8	20
11	1	2
Total	42	100

III *Standing*

Group	No. of subjects	%
0	5	12
1— 2	13	31
3—10	22	52
11	2	5
Total	42	100

In only 12% of the subjects was the ST T region unchanged, 31% had changes of types 1—2, 52 % changes of types 3—10. Here again there were few (5 %) who exhibited an ST region of type 11. The changes were most pronounced in leads II and CH (table III)

During exercise

Table 16 shows that lanatoside C in this dose caused more pronounced ST T changes than at rest and standing.

In only one subject was the ST T region unchanged. Changes of types 1—2 were recorded in 17 % of the subjects, types 3—10 in 74 % type 11 only in 7 %. The ST T changes were most pronounced in lead CH_7

IV *During exercise*

Group	No. of subjects	%
0	1	2
1—2	7	17
3—10	31	74
11	3	7
Total	42	100

After exercise

Immediately after exercise 60 % had unchanged ST T region, in 26 % digitalization caused changes of types 1—2, in 12 % more pronounced changes.

Three minutes after exercise the changes had become more accentuated, 29 % having changes of types 3—10 19 % of types 1—2, and 45 % no changes in the ST T region.

Table 13 shows the height of the T wave at rest and during the post-exercise period. At rest the T waves were throughout lower after digitalization, and likewise after exercise, the differences being highly significant ($p < 0.001$)

Prior to digitalization the T waves were higher immediately after exercise than at rest, but returned to the resting value 3 minutes after exercise. The same pattern was observed after digitalization, the T waves immediately after exercise being higher than at rest but 3 minutes after exercise again of the same height as at rest.

The same tendency is seen from tables V and VI. Immediately after exercise 26% exhibeted changes in group 1—2 and 12% in group 3—10, whereas 3 min. after exercise 19% had changes in group 1—2 and 29% in group 3—10.

V *Immediately after exercise*

Group	No. of subjects	%
0	25	60
1—	11	26
3—10	5	12
11	1	2
Total	42	100

VI *3 min after exercise*

Group	No. of subjects	%
0	19	45
1—2	8	19
3—10	12	29
11	3	7
Total	42	100

Table 13 Height of T wave in mm at rest and in the postexercise period (1 mm = 0 1 mV) before and after 1.6 mg Lanatoside C
(M±S.E., S.D.)

Lead	Rest		Immediately after exercise		3 min after exercise	
	before	after	before	after	before	after
I	2.9 ± 0.2 0.9	1.8 ± 0 14 0.9	3 2 ± 0.21 1.2	2.1 ± 0 17 1.0	2.5 ± 0.13 0.9	1.5 ± 0.16 0.9
	(n = 41)		(n = 36)		(n = 39)	
II	3.5 ± 0 21 1.8	1.8 ± 0.24 1.5	4.5 ± 0.36 2.1	3 1 ± 0.28 1.6	3.5 ± 0.24 1.5	2.2 ± 0.31 1.8
	(n = 41)		(n = 32)		(n = 34)	
CR2	8.9 ± 0.61 3.8	6.5 ± 0.61 3.7	11.6 ± 0 67 3 6	8.4 ± 0 60 3 4	9.0 ± 0.51 3.0	6 4 ± 0.54 3.1
	(n = 38)		(n = 32)		(n = 33)	
CR5	7 1 ± 0 42 [illegible]7	4.3 ± 0 49 3 1	9 14 ± 0 44 2.6	6.5 ± 0 49 2.7	6.9 ± 0.40 2.4	3.9 ± 0.42 2.2
	(n = 38)		(n = 31)		(n = 30)	
CR7	5 1 ± 0.25 1 6	[illegible]9 ± 0.28 1 8	5.6 ± 0.29 1 7	3.3 ± 0.35 1 9	4.3 ± 0 35 1.8	2.4 ± 0.28 1 7
	(n = 41)		(n = 32)		(n = 35)	

B. *Lanatoside C 0 4 and 0.8 mg*

Lanatoside C in doses of 0 4 mg intravenously changed the ST T region to a very much smaller extent than in doses of 1 6 mg

At rest two subjects showed changes of types 1—2 (n = 8) in standing position one subject had changes of type 3 and one of type 4

During exercise this small dose of lanatoside C caused ST T changes in four subjects (n = 8) in three of whom types 1—2 and in the fourth type 3 Immediately after exercise no ST T changes were recorded while 3 minutes after exercise one subject showed changes of type 2.

The changes in the ST T region caused by 0 8 mg of lanatoside C intravenously are set out in tables 18 19 20 and 21 The tendency is the same as after 1 6 mg, but the changes are perhaps slightly less pronounced. But all subjects in this group (n = 9) exhibited ST T changes during exercise which were not recorded before digitalization. The changes were most pronounced in lead CH_7 After exercise the ST T changes quickly receded.

C. *Digitoxin*

The types of ST T changes caused by digitoxin per os are shown in tables 22 23 24 and 25

At rest

At rest (table 22) the conditions were roughly similar to those after digitalization with 1 6 mg of lanatoside C 61 % of

the subjects showed no changes, 9 % had changes of types 1—2, and 13 of types 3—10. Changes of type 11 were observed in four subjects (17 %)

VII. *At rest*

Group	No. of subjects	%
0	14	61
1—2	2	9
3—10	3	13
11	4	17
Total	23	100

Standing

After digitoxin the ST T region changed in largely the same way as after lanatoside C. It will be seen from table 23 that the changes were more common and of a more pronounced kind than at rest.

No changes were shown by 30 % changes of types 1—2 by 18 % types 3—10 by 30 % type 11 by 22 %

VIII *Standing*

Group	No. of subjects	%
0	7	30
1—2	4	18
3—10	7	30
11	5	22
Total	23	100

D ring ercise

The results are presented in table 24 They do not differ appreciably from those for lanatoside C.

Digitoxin provoked changes within the ST T region in 91 % of the subjects, of types 3—10 in 69 %

IX. *During exercise*

Group	No. of subjects	%
0	2	9
1—2	5	22
3—10	16	69
11	0	
Total	23	100

After exercise

During the post-exercise period (table 25) the changes induced by digitoxin quickly receded. Neither immediately nor 3 minutes after exercise was there any ppreciable difference from the observa tions during the post-exercise period after lanatoside C.

The percentual distribution of ST T types shown in tables X and XI.

X. *Immediately after e ercise*

Group	No. of subjects	%
0	10	44
1—2	9	39
3—10	4	17
11	0	
Total	23	100

XI *3 minutes after exercise*

Group	No. of subjects	%
0	9	39
1—2	8	35
3—10	6	26
11	0	
Total	23	100

D *Digoxin*

The digoxin group comprises comparatively few subjects (n = 10) The results are shown in tables 26 27 28 and 29 The tendency is the same as after lanatoside C and digitoxin, the ST T changes being comparatively few at rest and more numerous in standing During exercise all digoxin subjects exhibited ST T changes.

Summary

Ninety two tests were made with different digitalis glucosides. Digitalis glucosides caused ST T changes at rest in 30 cases (32 %) standing in 69 cases (75 %) during exercise in 82 cases (89 %) Immediately after exercise the number of cases with ST T changes was 34 (37 %) and 3 minutes after exercise 44 (48 %) The types of changes are tabulated below (table XII)

Localization

It is quite clearly apparent from tables 14—29 that ST T changes most often occur in the left precordial leads and least often in the right precordial leads. The percentual distribution is shown in table XIII

XII

Type	Rest %	Standing %	During exercise %	Immediately after exercise %	3 minutes after exercise %
0	68	25	11	63	52
1— 2	13	25	27	25	21
3—10	14	42	58	10	23
11	5	8	4	2	4
Total	100	100	100	100	100

XIII.

Lead	Rest		Standing		Exercise		Post exercise			
							0 min		3 min	
	No	%	No	%	No	%	No	%	N	%
I	15	17	38	16	—	—	15	14	13	9
II	21	24	58	25	—	—	29	30	28	20
CR/CH	3	3	17	7	23	11	2	2	5	4
CR/CH_4	14	16	33	14	53	24	12	20	24	17
CR/CH_5	17	19	38	16	65	29	19	21	34	24
CR/CH	19	21	52	22	81	36	20	15	37	26
Total	89	100	236	100	222	100	95	100	141	100

Types and distribution of ST and T changes after administration of lanatoside C (1.6mg) *(n = 42)*

14 At rest



0	1	2	3	4	5	6	7	8	9	10	11
33	9										
28	3	2	7	1		1					
41				1							
37		1	1	2	1						
35	1	2	2	1							1
32	4	3	1				1				1

15 Standing

19	20		2								1
9	10	2	8	3	2	4	1	1			2
34	2		5	1							
26	3		8	1		1	1	1			1
22		2	4	5		2	1	1			1
14	9	3	3	5	1			1			4

16 During exercise

25	2	4	1	5			2				
14	4	3		7		1	10		2		
5		7		8		3	12		2	1	1
1		8		14		3	8		2	1	3

17 After exercise

0'	3'	0	3	0'	3	0	3'	0'	3'	0'	3	0'	3	0'	3	0'	5	0'	3	0'	3	0'	3'
35	35	3	2	2	2		3		3														
30	27		1	7	4	1	2	3	7				1									1	
42	40		1		1																		
35	27	1		5	6	1	1		6						1								5
33	24	1	1	7	4				8				3									1	2
35	22		1	5	5		1	3	7				3									1	3

Types and distribution of ST and T changes after administration of lanatoside C (0.8 mg)
(n = 9)

Table 18 At rest

lead	0	1	2	3	4	5	6	7	8	9	10	11	changes total
I	8	1											1
II	8			1									1
CR2	8				1								1
CR4	8				1								1
CR5	8		1										1
CR7	8		1										1

Table 19 Standing

I	5	3	1										4
II	2	1	1	4	1								7
CR2	6			2					1				3
CR4	5			2	1		1						4
CR5	4	1		2	1		1						5
CR7	1	3	1	4									8

Table 20 During exercise

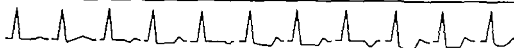
CH2	5	2		1	1								4
CH4	4	1	1	1	1			1					5
CH5	4	1	2	1				1					5
CH7	0	6	1	1				1					9

Table 21 After exercise

	0'	5'	0'	5'	0'	5'	0'	5'	0'	5'	0'	5'	0'	5'	0'	5'	0'	5'	0'	5'	0'	5'	0'	5'	0'	5'
I	9	7		2																						
II	8	7			1	1		1																		2
CR2	9	7				1		1																	1	2
CR4	9	7					2																			2
CR5	9	7					2																			2
CR7	9	6		2		1																				2
																										3

Types and distribution of ST and T changes after administration of digitoxin per os
(n = 23)

22 At rest



0	1	2	3	4	5	6	7	8	9	10	11
19	3										1
19		2									
22				1							
16		4	1								2
16	1	4									2
15		3	1	1							3

23 Standing

17	4		1								1
8	4	1	1	2	1	1					3
20	1	1					1				
15	1	3	1					1		1	
12	1	4	1	1		1		1			1
10	4	2	1		1			1			3

24 During exercise

18	1	1	1			1					
7		2	2	4		2	1		3	2	
6		1		3		3	6			2	
2		4		3		3	6		1	2	

25 After exercise

0'	3	0'	3	0'	3	0'	3'	0	3	0'	3	0'	3	0	3'	0'	3	0'	3	0'	3	0'	3	
17	19	3	1	1			1	2	1				1											
11	14			9	4	1			4				1											1
21	22			1	1															1			1	
19	16			3	3			1	1										1		1		1	
15	11			4	7	1			1				1		1					1	1		1	
13	11			8	5				2				3	2										1

Types and distribution of ST and T changes after administration of digoxin per os (n = 10)

Table 26 At rest

lead	0	1	2	3	4	5	6	7	8	9	10	11	changes total
I	9		1										1
II	8	1	1										2
CR2	9	1											1
CR4	9				1								1
CR5	8				1								2
CR7	9				1								1

Table 27 Standing

I	7	2			1								3
II	6	3			1								4
CR2	9	1											1
CR4	6	2		1	1								4
CR5	7	1		1			1						2
CR7	8	1					1						3

Table 28 During exercise

CH-	10												0
CH4	7		3										3
CH5	3		4		2							1	7
CH7	3	3			3							1	

Table 29 After exercise

lead	0'	5'	0'	5'	0'	5'	0'	5'	0'	5'	0'	5'	0'	5'	0'	5'	0'	5'	0'	5'	0'	5'	0'	5	0'	5'
I	9	9			1					1															1	1
II	8	8			1					1													1	1	2	2
CR2	10	10																								
CR4		9			2																		1		3	1
CR5	7	8			1									1									2	1	3	2
CR7	8	7			1	1								1									1	1	2	3

Types and distribution of ST and T changes after administration of digitoxin per os (n = 23)

' 22 At rest



d	0	1		3	4	5	6	7	8	9	10	11
	19	3										1
	19		2		?							
:2	22				1							
.4	16		4	1								2
.5	16	1	4									2
.7	15		3	1	1							3

e 23 Standing

	17	4		1								1
	8	4	1	1	2	1	1					3
:2	20	1	1					1				
:4	15	1	3	1					1		1	
:5	12	1	4	1	1		1		1			1
.7	10	4	2	1		1			1			3

e 24 During exercise

[2	18	1	1	1			1					
[4	7		2		4		2	1		3	2	
[5	6		1		3		3	6			2	
[7	2	?	4		3		3	6		1	2	

' 5 After exercise

	0'	3	0'	3	0'	3	0'	3	0'	3	0	3	0	3	0'	3	0'	3	0'	3	0'	3'	0'	3'	
	17	19	3	1	1			1	2	1				1											1
	11	14			9	4	1		2	4				1											
.2	21	22			1	1															1			1	
.4	19	16			3	3			1	1										1		1		1	
.5	15	11			4	7	1			1				1		1					1	1		1	1
.7	13	11			8	5				2				3	2										

Fig. 9.

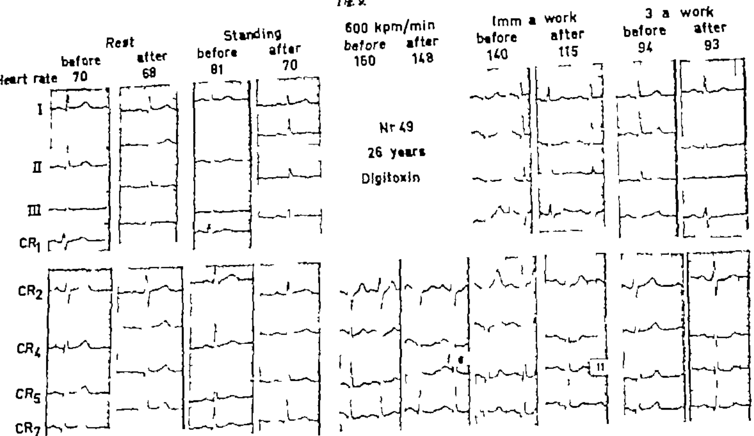

Rest
before
after
Standing
before
after
600 kpm/min
before
after
Imm a work
before
after
3 a work
before
after
Heart rate 70
68
81
70
160
148
140
115
94
93
I
II
III
CR_1
CR_2
CR_4
CR_5
CR_7
Nr 49
26 years
Digitoxin
11

Fig. 8. The figures on the ECG tracings in fig. 8, 9, 10 and 11 correspond to the types of ST and T changes shown in fig. 7

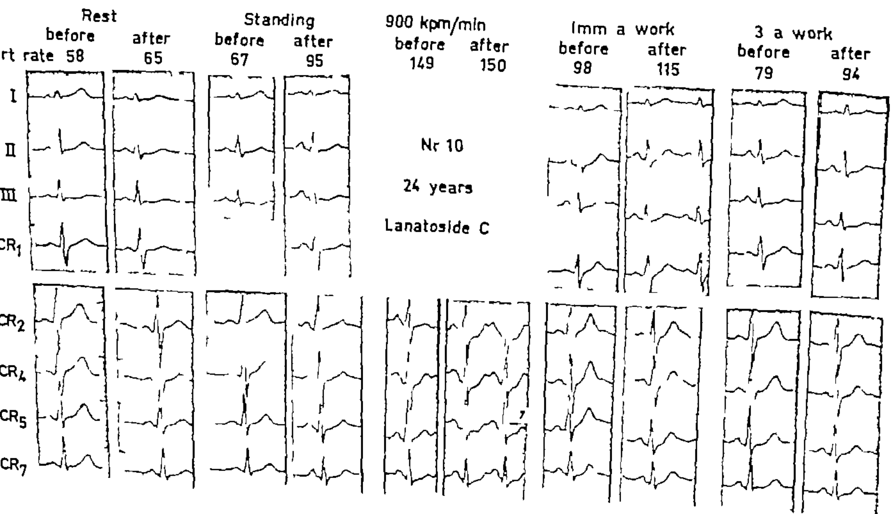


Fig. 11

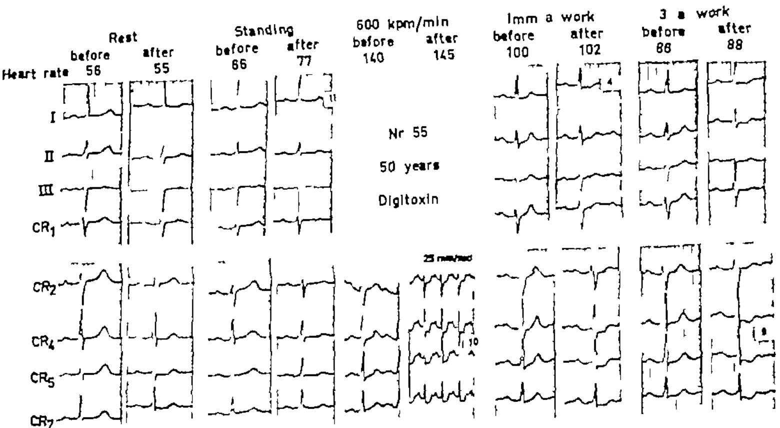
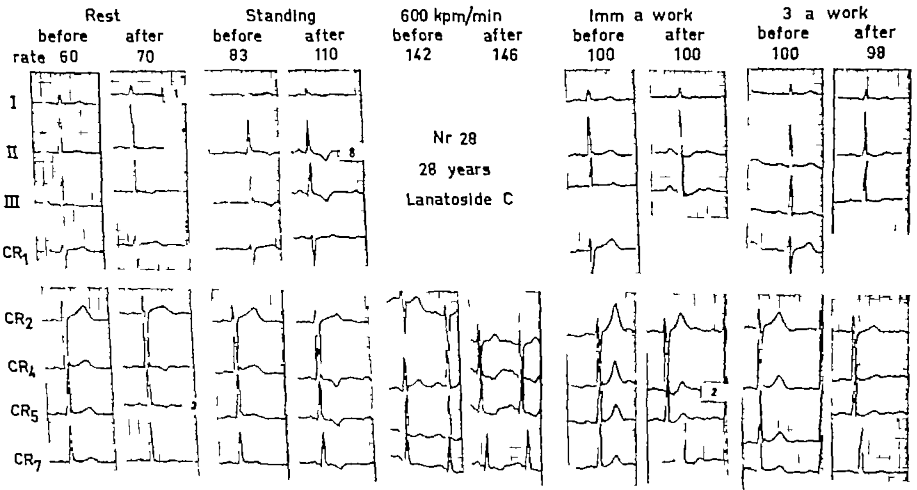

Rest
before
after
Heart rate
56
55
Standing
before
after
66
77
600 kpm/min
before
after
140
145
Imm a work
before
after
100
102
3 a work
before
after
86
88
I
II
III
CR_1
CR_2
CR_4
CR_5
CR_7
Nr 55
50 years
Digitoxin

Fig. 10


Rest
before
after
rate 60
70
Standing
before
after
83
110
600 kpm/min
before
after
142
146
Imm a work
before
after
100
100
3 a work
before
after
100
98
Nr 28
28 years
Lanatoside C
I
II
III
CR1
CR2
CR4
CR5
CR7
8
2

lis even in excessive doses (e. g. in attempted suicide) but A V block II with Wenckebach periods may occur (Hedley 1943 Bickel et al., 1951 Vuletic & Ivancic, 1952, MacMillan & Bellet, 1952)

Bengtsson (1957) found that during heavy exercise at a heart rate of around 170 per minute the P-Q interval dropped to the same level, viz. 0.11 second in all age groups (in his material up to 40 years) irrespective of the initial value at rest, or of age and sex.

After digitalis the P-Q interval was prolonged at rest and after exercise in my material there were isolated cases of A-V block I and one of A-V block II. At high loads (600 kpm/min for women, 900 kpm/min for men) the P-Q intervals were exactly the same as before digitalization, even in the subject with A V block II. Thus in healthy subjects digitalis had a moderate effect on the P-Q interval t rest and after exercise, but the P-Q interval during exercise was unaffected by digitalis. Nor was there other than low grade block in my material.

Total block has admittedly been produced in dogs after very large doses of digitalis (Moe & Mendez, 1952, Dearing & al., 1943) but clearly the doses used by healthy persons have been too small (although toxic) to produce block, which is liable to appear as a sign of intoxication in the diseased heart

Th Q T terval

Handbooks report a shortening of the Q T interval as a constant finding in cases of digitalization (g Lepeschkin, 1951 Goldberger 1953 Gardberg, 1957) Cheer & Dieuaide (1931) showed that digitalis shortened the Q T interval and they considered this actually to be the most sensitive indicator of digitalization. Larsen & Nielsen (1936, 1937) Wedd & Blair (1947) Sandberg et al. (1950) MacMillan & Bellet (1952) and Rothlin (1954) also found that digitalis definitely shortened the Q-T interval. Rothlin says that this is the first objective sign of the effect of digitalis, and MacMillan & Bellet that it may be a good way of differentiating ST changes caused by coronary insuffiency from those caused by digitalis.

Doubt on this score has been expressed from several quarters. Geiger et al (1941) found shortening of the Q-T interval in some cases, but of an extremely temporary nature. Ruskin & Décherd (1948) found in animal experiments that small doses prolonged the Q-T interval while large doses shortened it. Koelbing (1950) in human experiments, found that small doses had no effect on the Q-T interval, while large doses produced fairly transient changes. Dönhardt (1951) reported a shortening of the Q-T interval in roughly half of his subjects.

Yu et al. (1952) found no direct shortening of the Q-T interval, but a lowering of the QT/TQ ratio at rest though none during exercise. These are the only authors to have recorded the ECG during exercise. Woodbury & Hecht (1952) found that cardioactive glucosides had no effect on the depolarization of the frog heart but shortened the repolarization time, which would explain the shortening of the Q-T interval.

As was stated in the section on measuring methods, measurement of the Q-T interval is difficult. After for example, tilting and exercise the positive after potential increases (Sjöstrand, 1951) This happens also after digitalis, as I found in my experiments. This positive after potential, which is added to the T wa e, makes it extremely difficult to decide when the T wave ends, especially at high frequencies. I am aware that the use of an index, i. e. introducing still another measurement,

Discussion

Irregular or ectopic impulse formation and change of impulse conduction have have been observed in patients in conjunction with more or less pronounced digitalis intoxication sinus arrhythmia, wandering pacemaker sino-auricular standstill, nodal rhythm, atrio-ventricular dissociation A V block of all degrees, premature beats see *inter alia* Burwell & Hendrix, 1950 Pick 1957

The effect on the heart rate has been dealt with earlier and will therefore be disregarded here

Sinus arrhythmia following upon digitalization has been regarded as may be a sign of light digitalis intoxication (Bellet, 1963) Larsen & Nielsen (1936 1937) found some cases of sinus arrhytmia after digilatization of healty subjects.

In my series sinus arrhythmia induced by digitalis was recorded at rest in one third of the subjects, but in none during exercise. During the post-exercise period only 3 had sinus arrhythmia.

Among other rhythmic disorders in the sino-auricular node sino-atrial block was observed in one case immediately after exercise Liebow & Feil (1941) found two cases of sino-atrial block in healthy digitalized persons immediately after exercise In cardiac patients sinoatrial block has been recorded in conjunction with digitalis intoxication (Flaxman, 1948 Herrman et al 1944 Burwell & Hendrix, 1950)

In my material occasional supraventricular premature beats were recorded after digitalis, and only a few ventricular premature beats, of which some during exercise at the lightest load. No premature beats were recorded at higher loads Case 15 who had ventricular premature beats even before digitalization, was not included in table 12 This person had ventricular premature beats at rest, standing and during exercise except at the heaviest load Ventricular premature beats returned some time after exercise. Digitalization did not increase the frequency of premature beats. This person was and is completely healthy but was a heavy smoker He was re-examined six months later when he had stopped smoking, and then exhibited no ventricular premature beats either before or after digitalization.

In persons with healthy hearts premature beats, particularly ventricular are extremely rare after digitalization, even in toxic doses (MacMillan & Bellet, 1952, Sagall & Wolff 1949 Larsen & Nielsen, 1936 Hedley 1943) In persons who have taken enormous doses of digitalis for suicidal purposes, only occasional or no premature beats have been recorded (Bachman, 1960 Bickel et al., 1951 Vuletic and Ivancic 1952 Nordström—Öhrberg 1962) Yu et al. (1952) found two cases of ventricular premature beat after exercise in healthy subjects in conjunction with digitalis during exercise no premature beats were recorded. Moe & Mendez (1951) showed, howerer that ventricular arrhythmia could be provoked in dogs with doses 70—80 % of the lethal dose (therapeutic doses are around 35 % of the lethal dose)

The *A V conduction* is varyingly affected by digitalis, from no effect at all (Apter et al. 1944 Koelbing 1950 Geiger et al. 1941 Yu et al 1952) prolongation of P-Q interval (Larsen & Nielsen, 1936, Sagall & Wolff 1949 Ruskin & Décherd, 1948) to block of mild degree, i.e. A V block I and A V block II with Wenckebach periods (Gilchrist, 1958) These low grade blocks diminish after exercise, while high grade blocks (A V block II with constant conduction time and A V block III) increase after exercise (Gilchrist, 1958 Landegren, 1964) In healthy persons total block never occurs after digita

23 % displaying no change. The percentual distribution of the changes was usually similar for the different glucosides. The standing heart rate was higher than the supine. In the lanatoside C cases, moreover the heart rate rose still further after digitalization. The standing ST T reaction was, however fairly similar for all three glucosides despite the difference in standing heart rate between the lanatoside C cases on the one hand and the digitoxin and digoxin on the other The rise of heart rate from supine to standing position may have been sufficient to accentuate the existing ST T changes and provoke new ones. But it would be expected that the lanatosid C subjects, who had a higher standing heart rate than the other subjects, would display more changes.

During exercise the ST T depression increased still further 89 % of the subjects showing changes after digitalization, and 58 % of types 3—10 Immediately after exercise the height of the T waves increased and the ST T changes decreased. Three minutes after exercise the ST T changes increased again.

Sjöstrand (1950 a and b) reported that the amplitude of the T wave normally decreases and the ST depression increases with rising heart rate and that the decrease in T-wave during work is just the reverse of the effect observed in the horizontal position immediately after the cessation of work Between one and two minutes after the cessation of work, however the amplitude of T-wave again decreases.

It is reasonable to suppose, therefore that major reason for the heavy accentuation of the post-digitalization ST T changes during exercise is the rise of heart rate This sympathicotonic element in the electrocardiographic picture is evident also in the circumstance that both ST and T regions in this material were normalized immediately after exercise. This is in agreement with the observations in cases of high sympathetic tone (Holmgren et al. 1957 1959) To regard the electrocardiographic changes induced by digitalis as *solely* due to increased sympathetic tone, on the other hand, is hardly realistic. Koelbing (1950) for example, proved that digitalis-induced changes are not affected by ergotamine as are the T-wave changes of sympathicotonic origin (Nordenfelt 1941 Björck 1947)

Liebow & Feil (1941) found that 4 digitalized subjects of 14 in the post-exercise period displayed ST T changes which were considered pathological though without specification of their degree and Yu et al. (1952) that 7 of 20 subjects during exercise and in the post-exercise period had pathological ST T changes after digoxin, but again without characterization of type.

The changes on the ECG particularly in the ST T region, produced by cardioactive glucosides have occasionally been considered to be proportional to the degree of digitalization. On this assumption attempts have been made to use the ECG for human bioassay (Pardee 1923, Gold et al. 1941 Tandowsky et al 1944) found the electrocardiographic criteria of the effect of digitalis fully reliable, and Wood (1956) writes The electrocardiogram offers by far the most reliable evidence of digitalis saturation

As early as 1941 Geiger et al. concluded "that the electrocardiogram has no practical clinical value for the quantitative estimation of the digitalis saturation of patients and this is the current view (de Graff 1946, Koelbing 1950 Dönhardt 1951 Simon 1962, and others)

From the present experiments it is evident that the ST T changes after 0.4 mg lanatoside C were extremely small, and that after 0.8 mg lanatoside C intraven-

may affect the result still more. But the use of an index is simple and practical, and there is no particular advantage in calculating the corrected Q T interval in accordance with one of the customary formulae.

In my experiments the index" was lower at rest i.e. the Q T interval was shorter after digitalis medication as also observed earlier at rest. On the other hand digitalization did not shorten the Q-T interval in the standing position nor during or after exercise. Why this should be so is difficult to explain.

But shortening of the Q-T interval is of little value in diagnosing the effect of digitalis in clinical practice, for one usually has no knowledge of the Q-T interval before digitalization. "Normal" Q T intervals vary within wide limits. Neither in the literature referred to in this paper nor in my experiments was the shortening of the Q T interval (when it occurred at all) of such magnitude as to be a conspicuous feature.

The ST and T region

Cohn & Fraser reported in 1913 T wave changes in the electrocardiogram as a result of digitalization. Since then the literature has abounded with accounts of the effect of digitalis on the ST T region, both in heart patients (Pardee 1923 Strauss & Katz 1934 Luten 1936 Stewart & Watson 1938 Eichna et al. 1943 Tandowsky et al. 1944 Rothlin 1954) and in healthy persons (Routier & Puddu 1935 Larsen & Nielsen 1936 1937 Geiger et al. 1941 Koelbing 1950 Donhardt 1951 Beers et al. 1951 MacMillan & Bellet 195? and Tilhakos 1953) Routier & Puddu (1935) found that changes in the ST T region occur as often in healthy as in cardiac patients.

All these studies have related to the ECG at rest, and almost all authors state that changes within the ST T region caused by digitalis cannot be distinguished from those seen in coronary insufficiency

Attempts have been made to arrive at a qualitative basis for diagnosing ST T changes as due to digitalization and also to grade the changes (Lepeschkin 1951 MacMillan & Bellet 1952, Goldberger 1953) MacMillan & Bellet described different types of changes which they classified in four grades from slight T wave depression to very pronounced ST T depressions. One type of change was a depressed, smoothly concavely rounded, "cup-shaped" ST segment. It is this form which is often regarded as typical of digitalization. Goldberger (1953) reported this form to be comparatively rare. In the present study it is classified as type 11 and appears in only a few per cent of all cases. The usual reference in the literature, however is to typical sagging of the ST segment.

Yet it is difficult to indicate any particular change as typical of digitalis glucosides. This is apparent from the findings in this study in which eleven types are described. In the author's opinion it is more reasonable to speak of nonspecific ST T changes for as Friedberg & Zager (1961) state, "although characteristic ST T wave changes have been described following digitalis, the changes are not always characteristic"

In the present study the ST T changes at rest were comparatively slight, 68 % of subjects displaying no change 13 % having changes of types 1—2, 14 % more pronounced changes, and only 5 % showing the concave cup-shaped type referred to above.

In standing position the changes within the ST T region were more common and more pronounced, 42 % of all subjects having changes of types 3—10 and only

In the present study G was determined at rest and standing before and after digitalis. The results are shown in table 30.

As is seen, AQRS was unchanged after digitalis, which accords with the fact that digitalis glucosides do not affect the depolarization. Likewise the direction of the vector ÂQRS shows no statistically significant changes. G on the other hand, declines, being 8.4 units after digitalization against 11.7 before, a highly significant difference ($p < 0.001$). Before digitalis G was smaller standing than prone. After digitalis it decreased still further. Before digitalis G was 7.6 units in standing, after digitalis 6.0 units, a probably significant difference ($p = 0.02$—0.01).

The direction of G showed only slight variations, the differences before and after digitalization being non-significant ($p > 0.1$).

The magnitude of G is influenced by many non-pathological factors. Apter et al. (1944) showed that digitalis reduced the gradient but did not change its direction. A high heart rate likewise reduces the gradient (Rosen 1957). The reduction of the gradient causes a shift of the ST segment. The combination of digitalis and high heart rate means that one must usually count on ST shifts due to diminution of the magnitude of the gradient under these conditions.

In ischaemic heart disease, on the other hand, the gradient is both diminished and its direction is changed, as shown in conjunction with positive hypoxaemia tests by Biörck et al. (1948).

Table 30

	No. of subjects	AQRS units	ÂQRS degrees	G units	Ĝ, degrees
rest, before digitalis	27	5.5 ± 0.34 2.9	+58 ± 5.4 28	11.7 ± 0.62 3.3	+45 ± 4.6 24
rest, after digitalis	27	5.5 ± 0.54 2.8	+61 ± 4.2 22	8.4 ± 0.64 3.4	+49 ± 3.4 18
standing before digitalis	27	4.3 ± 0.44 2.3	+61 ± 10.6 55	7.6 ± 0.65 3.4	+43 ± 4.7 23
standing, after digitalis	27	4.3 ± 0.47 2.4	+62 ± 9.4 47	6.0 ± 0.37 1.9	+39 ± 5.9 30

ously and 1 25 mg digoxin orally the changes were rather more pronounced and more frequent. After 1 6 mg lanatoside C and after digitoxin the changes were more common. On the other hand changes occurred after very varying doses, and subjects who had subjective symptoms after digitalization did not exhibit more pronounced changes than the asymptomatic subjects. So far as can be judged from this material it is impossible to decide the degree of digitalization on the basis of the electrocardiogram.

There has been much controversy about the genesis of the ST T changes. The apparent similarity between ST changes caused by digitalis and those seen in coronary disease has earlier been regarded as an indication that digitalis really causes a decline of the coronary blood flow (Büchner 1934 Levy et al 1940 Sandberg et al 1950) This was thought to lead to cardiomuscular necrosis which in animal experiments has been observed after massive doses of digitalis (Büchner 1933 1934 Bauer 1934 Dearing et al 1943b) These doses were, however many times larger than therapeutic doses. after therapeutic doses these changes were not observed. Neither Dearing et al. nor La Due (1941) found any changes in the heart muscle of dogs which had been given a full digitalization dose daily for one month.

In fact it has been convincingly proved that the coronary blood flow is not diminished by digitalis (Bing et al. 1950 Bing & Daley 1951 Wegria 1951) Exercise tests of the kind described in this study do not indicate any impairment of the myocardial function.

Woodbury & Hecht (1952) using a microelectrode technique, studied the effect of digitalis glucosides on the frog heart in situ They found that cardioactive glucosides did not affect the depolarization but did affect the repolarization, the duration of the membrane action potential being markedly shortened. Surface electrocardiograms recorded from the ventricular surface exhibited ST changes which could be correlated to simultaneous single fibre records. The *uniform* shortening of the repolarization thus resulted in depression of ST and shortening of QT

The effect of cardioactive glucosides on the various metabolic processes and on the sodium and potassium concentration, by which the action potential is ultimately affected, lie outside the scope of the present study

The changes occurred principially in the left precordial leads, which conforms with previous reports. Beers et al. (1951) found the most pronounced changes from digitalis in the left precordial leads for horizontal hearts and in the right precordial leads for vertical hearts. MacMillan & Bellet (1952) state that the largest ST T depressions in conjunction with digitalization occur in the leads where R is greatest, and Paul Wood (1956) sums up by saying that the ST T changes are most pronounced in leads V 4—6.

Ventricular gradient

The concept of the ventricular gradient was created by Wilson and later developed by Ashman (1943 Ashman & al. (1943 a and b) and Gardberg & Ashman (1943)

The ventricular gradient (G) represents in the frontal plane the projection of the net electrical effects of differences in rates of repolarization in various areas of the ventricular muscle.

There has been controversy about the use of G since G varies enormously from individual to individual and even *within* the same individual. But it has been stated that G is of value for the study of serial electrocardiograms (Gardberg 1957)

In the present study G was determined at rest and standing before and after digitalis. The results are shown in table 30.

As is seen, AQRS was unchanged after digitalis, which accords with the fact that digitalis glucosides do not affect the depolarization. Likewise the direction of the vector ÂQRS shows no statistically significant changes. G, on the other hand, declines, being 8.4 units after digitalization against 11.7 before, a highly significant difference ($p < 0.001$). Before digitalis G was smaller standing than prone. After digitalis it decreased still further. Before digitalis G was 7.6 units in standing, after digitalis 6.0 units, a probably significant difference ($p = 0.02—0.01$).

The direction of G showed only slight variations, the differences before and after digitalization being non-significant ($p > 0.1$).

The magnitude of G is influenced by many non-pathological factors. Apter et al. (1944) showed that digitalis reduced the gradient but did not change its direction. A high heart rate likewise reduces the gradient (Rosen 1957). The reduction of the gradient causes a shift of the ST segment. The combination of digitalis and high heart rate means that one must usually count on ST shifts due to diminution of the magnitude of the gradient under these conditions.

In ischaemic heart disease, on the other hand, the gradient is both diminished and its direction is changed, as shown in conjunction with positive hypoxaemia tests by Biörck et al. (1948).

Table 30

	No of subjects	AQRS, units	ÂQRS degrees	G, units	Ĝ, degrees
rest, before digitalis	2	5.5 ± 0.54 2.9	+58 ± 5.4 28	11.7 ± 0.62 3.3	+43 ± 4.6 24
rest, after digitalis	27	5.5 ± 0.54 2.8	+61 ± 4.2 22	8.4 ± 0.64 3.4	+49 ± 3.4 18
standing, before digitalis	27	4.5 ± 0.44 2.3	+61 ± 10.6 53	7.6 ± 0.65 3.4	+43 ± 4.7 23
standing, after digitalis	27	4.5 ± 0.47 2.4	+62 ± 8.4 47	6.0 ± 0.3 1.9	+39 ± 5.9 30

ously and 1 25 mg digoxin orally the changes were rather more pronounced and more frequent. After 1 6 mg lanatoside C and after digitoxin the changes were more common. On the other hand changes occurred after very varying doses and subjects who had subjective symptoms after digitalization did not exhibit more pronounced changes than the asymptomatic subjects. So far as can be judged from this material, it is impossible to decide the degree of digitalization on the basis of the electrocardiogram.

There has been much controversy about the genesis of the ST T changes. The apparent similarity between ST changes caused by digitalis and those seen in coronary disease has earlier been regarded as an indication that digitalis really causes a decline of the coronary blood flow (Büchner 1934 Levy et al 1940 Sandberg et al 1950) This was thought to lead to cardiomuscular necrosis which in animal experiments has been observed after massive doses of digitalis (Büchner 1933 1934 Bauer 1934 Dearing et al 1943b) These doses were, however many times larger than therapeutic doses after therapeutic doses these changes were not observed. Neither Dearing et al nor La Due (1941) found any changes in the heart muscle of dogs which had been given a full digitalization dose daily for one month.

In fact it has been convincingly proved that the coronary blood flow is not diminished by digitalis (Bing et al. 1950 Bing & Daley 1951 Wegria 1951) Exercise tests of the kind described in this study do not indicate any impairment of the myocardial function.

Woodbury & Hecht (1952) using a microelectrode technique studied the effect of digitalis glucosides on the frog heart in situ. They found that cardioactive glucosides did not affect the depolarization but did affect the repolarization, the duration of the membrane action potential being markedly shortened. Surface electrocardiograms recorded from the ventricular surface exhibited ST changes which could be correlated to simultaneous single fibre records. The uniform shortening of the repolarization thus resulted in depression of ST and shortening of QT

The effect of cardioactive glucosides on the various metabolic processes and on the sodium and potassium concentration, by which the action potential is ultimately affected, lie outside the scope of the present study

The changes occurred principally in the left precordial leads, which conforms with previous reports. Beers et al. (1951) found the most pronounced changes from digitalis in the left precordial leads for horizontal hearts and in the right precordial leads for vertical hearts. MacMillan & Bellet (1952) state that the largest ST T depressions in conjunction with digitalization occur in the leads where R is greatest, and Paul Wood (1956) sums up by saying that the ST T changes are most pronounced in leads V 4—6

Ventricular gradient

The concept of the ventricular gradient was created by Wilson and later developed by Ashman (1943 Ashman & al. (1943 a and b) and Gardberg & Ashman (1943)

The ventricular gradient (G) represents in the frontal plane the projection of the net electrical effects of differences in rates of repolarization in various areas of the ventricular muscle.

There has been controversy about the use of G, since G varies enormously from individual to individual and even within the same individual. But it has been stated that G is of value for the study of serial electrocardiograms (Gardberg 1937)

Table 32. Duration of ST T changes after administration of digitoxin per os.

	<1 week	1 week	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks	Number of subjects
at rest	2	3	1	1		2	0	9
standing	4	4	2	5		1		16
during exercise	0	2	4	6	2	5	2	21
1 min. after exercise	0	2	3	2	2	3	1	13
3 min. after exercise	2	2	1	3	2	3	1	14

B. *Digitoxin*

The duration of ST T changes induced by digitoxin is shown in table 32.

Changes at rest were recorded in 9 subjects, and in 2 of them were still present five weeks after completion of digitoxin medication.

In standing position changes were recorded in 16 subjects, persisting for up to three weeks and in one case up to five weeks. In the 21 subjects exhibiting ST T changes during exercise, the changes were still present in some cases six weeks after medication. In a check-up of these subjects some eight weeks after medication their ECG had returned to the picture before digitalization.

After exercise the duration of ST T changes was roughly the same as during exercise. One subject still exhibited *changes six weeks after the end of medication.*

C. *Digoxin*

Ten subjects received a single dose of digoxin per os. The duration of the changes in the ST T region is shown in table 33.

The changes at rest persisted for less than one day. In standing position they lasted up to three days, and during and after exercise up to four days.

Table 33. Duration of ST T changes after administration of 1.25 mg digoxin per os.

	<1 day	1 day	2 days	3 days	4 days	5 days	Number of subjects
at rest	2	0				0	2
standing	2	1	1	1			5
during exercise	0	0	2	4	1		7
1 min. after exercise	1			1	1		3
3 min. after exercise		1		1	1	0	3

Duration of ECG changes

As noted earlier from table 12 30 subjects developed sinus arrhythmia after administration of digitalis, 15 of whom had had lanatoside C, 12 digitoxin and 3 digoxin. The arrhythmia was of comparatively short duration, lasting less than one day in 9 of the lanatoside C cases, one day in 3 of them and two days in 3

The sinus arrhythmia induced by digitoxin remained for less than one week in 8 subjects and for one week in 4 In the 3 digoxin cases the arrhythmia lasted less than one day one day and two days, respectively The premature beats recorded after digitalization were few in number and of occasional character One subject developed sino-atrial block after lanatoside C, lasting two days. A fairly pronounced prolongation of the P-Q interval was recorded in 7 subjects after lanatoside C, persisting for less than one day in 2, one day in 1 two days in 1 and four days in 3

After digitoxin, A V block II with Wenckebach periods lasting two weeks, was recorded in one subject after which the P-Q interval was considerably prolonged the prolongation persisting for three weeks. In another subject prolongation of the P-Q interval was recorded which persisted for less than one week.

The most common and important changes occurred, as noted earlier within the ST T region.

A. *Lanatoside C*

The duration of changes within the ST T region caused by 1 6 mg of lanatoside C is shown in table 31

At rest the changes persisted in some subjects for up to one day standing up to three days, during and after exercise up to five days.

Table 31 Duration of ST T changes after administration of 1.6 mg lanatoside C intravenously

	<1 day	1 day	2 days	3 days	4 days	5 days	6 days	Number of subjects
at rest	11	4	0					15
standing	13	16	7	1	0			37
during exercise	3	1	13	17	6	1	0	41
Imm. after exercise	5	2	4		2	2	0	17
3 min. after exercise	9	2	3	4	3	2	0	23

Table 32 Duration of ST T change after administration of digitoxin per os.

	<1 week	1 week	2 eeks	3 week	4 weeks	5 weeks	6 weeks	Number of subjects
t rest	2	3	1	1		2	0	9
standing	4	4	2	5		1		16
during exercise	0	2	4	6	2	3	2	21
1min. after exercise	0	2	3	2	2	3	1	13
3 min. after exercise	2	2	1	3	2	3	1	14

B. *Digitoxin*

The duration of ST T changes induced by digitoxin is shown in table 32.

Changes at *rest* were recorded in *9* subjects, and in 2 of them were still present five weeks after completion of digitoxin medication.

In standing position changes were recorded in 16 subjects, persisting for up to three weeks and in one case up to five weeks. In the 21 subjects exhibiting ST T changes during exercise, the changes were still present in some cases six weeks after medication. In check-up of these subjects some eight weeks after medication their ECG had returned to the picture before digitalization.

After exercise the duration of ST T changes was roughly the same as during exercise. One subject still exhibited changes six weeks after the end of medication.

C. *Digoxin*

Ten subjects received a single dose of digoxin per os. The duration of the changes in the ST T region is shown in table 33.

The changes at rest persisted for less than one day. In standing position they lasted up to three days, and during and after exercise up to four days.

Table 33 D ration of ST T kanges after administration of 1.25 mg digoxin per os.

	<1 day	1 day	2 days	3 days	4 days	5 days	Number of subjects
rest	2	0				0	2
standing	2	1	1	1			5
during exercise	0	0	2	4	1		7
1min. after exercise	1			1	1		3
3 min. after exercise		1		1	1	0	3

Discussion

In speaking of the duration of the effect of the various cardioactive glucosides one must distinguish between the *clinical effect* i e. how long a clinical effect persists in respect of ventricular rate during atrial fibrillation or of recurrence of incompensation and *the effect on the electrocardiogram* The duration of the clinical effect of digitoxin has been reported as 14—21 days, of lanatoside C and digoxin 3—5 days (Bellet 1963) Other authors report somewhat different durations. *Lown & Levine (1954) put the* duration of the digitoxin effect at more than 3 weeks, digoxin 4—7 days, and lanatoside C 2—3 days, while Hedlund (1952) gives a figure of up to 8 days for the effect of lanatoside C.

The duration of the effect on the ECG has earlier been studied only on the resting ECG Campbell (1942) stated that A V block caused by folium digitalis passed off quickly whithin 2—3 days, and Bachman *(1960) found that A V block* disappeared within a few days In the present study A V block persisted after digitoxin medication in one subject for two weeks.

ST T changes after folium digitalis have been reported to persist for one month (Larsen & Nielsen 1936 and Geiger et al. 1941) ST T changes caused by 1 6 mg lanatoside C disappeared within 16 hours (Tandowsky 1942) while the same author found that ST T changes caused by digitoxin disappeared in two days. ST T changes in conjunction *with attempted* suicide with digitoxin disappeared after 11 days (Bickel et al. 1961) After digoxin ST T changes at rest persisted for one to two days (Helander & Östman 1960)

The duration of ST T changes is extremely individual particularly as regards the slow-acting glucoside, digitoxin, in the present study Their duration at rest varied from less than one week to five weeks during and after exercise the duration *was in some cases even longer*

These periods agree fairly closely with the period reported for excretion of digitalis which is short for lanatoside C (Bine et al 1952) and longer for digitoxin. Friedman et al. (1950) demonstrated by biological methods that digitoxin, when given in a single dose, was excreted with the urine during 12—24 days.

Okita et al (1953) and Okita (1957) using radioactive digitoxin, found that *60—80 per cent was excreted* through the kidneys, and that during a period of up to 40 days (range 23—50 days) the excretion occurred in the form of unchanged digitoxin while metabolites were still being excreted some 80 days after the single dose. This is entirely in accordance with electrocardiographic observations of the duration of the ST T changes.

Abdon N.-O. Hammarskjöld S. O. and *Nielsen N. A.*, Skandinav. Arch. f. Physiologie 78 8, 1938.

Abdon, N.-O. and *Nielsen, N.* Skandinav. Arch. f. Physiologie 78 1 1938.

Apter L., Ashman R. and *Hull E.* J. Pharmacol. & Exper. Therap. 82 227 1944.

Ashman, R. Am. Heart J. 26 495 1943.

Ashman R., Byer E. Bayley R. H. Am. Heart J. 25 16 1943 a.

Ashman, R., Gardberg, M. and *Byer E.* Am. Heart J. 25 36, 1943 b.

Ashman R. Gardberg, M. and *Byer E.* Am. Heart J. 26 473 1943

Beckman M. Ztschr. Kreislaufforsch. 49 982, 1960

Barker S. B. and *Summerson, W. H.* J. Biol. Chem. 138 535 1941

Bauer H. Arch. exper. Path. Pharmacol. 176 65 1934

Beer R. Regan W. and *Jansen J.* Am. Heart J. 41 115 1951

Bellet S. Clinical Disorders of the Heart Beat, Lea and Febiger Philadelphia 1963

Bengtsson E. Acta med. scandinav. 154 21 1956 a.

Bengtsson E. Acta med. scandinav. 154 91 1956 b

Bengtsson E. Acta med. scandinav. 154 225, 1956 c.

Bengtsson E. Studies on the Electrocardiogram and Working Capacity Stockholm 1957

Beckel G. Plattner H. and Edelstein, H. Arch. mal. coeur 44 61 1951

Bine R. St. George S. and *Friedman M.* Proc. Soc. Exper. Biol. & Med. 79 513 1952.

Bing R. J. and *Daley R.* Amer. J. Med. 10 711 1951

Bing R. J. Maraist F. M. Dammann J. J. F. Draper J. A., Heimbecker R., Daley R. Gerard R. and *Calazel, P.* Circulation 2 513 1950.

Boberck, G. Brit. Heart J. 9 181, 1947

Boberck, G. Jackson F. S. and *Rohlin S.* Acta med. scandinav. 132 283 1948.

Bjur A. and *Lau E. H.* Upsala Läk. förh. 33 1 1927

Borison, H. L. J. Pharm. & Pharmacol. 104 396, 1952.

Borison H. L. and *Wang S. C.* Proc. Soc. Exper. Biol. & Med. 76 : 335 1951

Braunwald E., Bloodwell R. D., Goldberg L. I. and *Morrow A. G.* : J. Clin. Invest. 40 52, 1961

Burwell B. and *Hendrix J. P.* Am. J. Med. 8 640, 1950.

Büchner F. Klin. Wchnschr. 55 1237 1933.

Buchner F. Arch. exper. Path. u. Pharmacol. 176 59 1934.

Campbell, M. Brit. Heart J. 4 131 1942.

Cannon, P. and *Sjöstrand, T.* : Acta med. scandinav. 146 191 1953.

Carlson L. A. and *Persson B.* Acta med. scandina. 164 39 1959

Cattell McKeen and *Gold H.* J. Pharmacol. & Exper. Therap. 62 116, 1938.

Chen S. N. and *Dunside F. R.*: Chinese J. Physiol. 5 217 1931

Cohen, M. B. New England J. Med. 246 225 1952 a.

Cohen B. M. New England J. Med. 246 254 1952 b.

Cohn A. E. and *Fraser F. R.* J. Pharm. & Pharmacol, 5 512, 1913.

Cohn A. E. and *Stewart H. J.* J. Clin. Invest., 6 53 1928.

Cotts de V. M. and Stopp, Ph. E. Am. J. Physiol. 192 114 1958.

Dearing, W. H. Barnes A. R. and *Essex H. E.* Am. Heart J. 25 665 1943 a.

Dearing, W. H. Barnes A. R. and *Essex, H. E.*, Am Heart J. 25 648, 1943 b.

DeGraff A. New York J. Med. 46 1803 1946.

Dock W. and *Tainter M. L.* J. Clin. Invest. 8 467 1930.

Dr sdale D. T. Yacoub T. Z., Michlom R. J. Schultz M. and *Lu ger M.*: Am. J. Cardiol. 4 88, 1959

Dönhardt A Ztschr Kreislaufforsch, 40: 548 1951

Eichna L. W Taube H and *DeGraff A* J Pharmacol. & Exper Therap. 78 22 1943

Erickson E W and *Fahr G E.* Am. Heart J 29 318 1945

Fisher R A Statistical Methods for Research Workers Oliver and Boyd Edinburgh, 1950.

Flaxman N Am. J M. Sc. 216 179 1948.

Friedberg Ch. K. and *Zager A* Circulation 23 633 1961

Friedman M B ne R., Byers S O and *Bland C* Circulation 2 749 1950.

Gardberg M Clinical Electrocardiography Harper & Brothers New York 1957

Gardberg M and *Ashman R.* Arch. Int. Med. 26 84? 1943

Geiger A J Blaney L F and *Druckemiller W H* Am. Heart J 22 230, 1941

Gilchrist A R Scottish M J 3 53 1958.

Gold H Fed Proc. 3 72 1944

Gold H Cattell M Kwit N and *Kramer M* J Pharmacol. & Exper Therap. 73 212 1941

Gold H Kwit N T Cattell McKeen and *Travell J* J A. M. A. 119 928 1942.

Gold H C ttell McKe Modell W G er-ner T and *Gueva R.* J Pharm. & Pharmacol. 98 337 1950.

Gold H Greiner T Cattell M Modell, W Gluck J Marsh R Mathes S Hudso D Robertson R. Warshaw L. Otto H Kwit N and *Kramer M* Am. J Med. 13 124 1952.

Gold H Kwit N T., Otto H and *Fox T* J Clin. Invest. 18 429 1939

Gold H Cattell McKeen Kwit N T and *Kramer M* J Pharmacol. & Therap. 73 212 1941

Goldberger E. Unipolar Lead Electrocardiography and Vectorcardiography *Lea & Febiger Philadelphia*, 1953

Gremels H Arch. exper Path. u. Pharmakol. 179 360 1935

Green K. E. Som Supplementary leads in Clinical Electrocardiography Stockholm 1948.

Harvey R M Ferrer I Carthcart R T and *Alexander J K* Circulation 4 366 1951

Hatcher R. A and *Weiss S* Arch. Int. Med. 29 690, 1922

Hedlund S. Cardiologia 20 *suppl ad, 1952.*

Helander S and *Östman J* Svenska läk. tidn. 57: 3706 1960

Hedley O F Ann. Int. Med *18* 154 1943

Hellström R. Acta med. scandinav Suppl. 371 1961

Herrman G R., Decherd G M and *McKinley W F J A M A 18 760 1944*

Heymans J F and *Heymans C* J Pharm. & Pharmacol. 29 203 *1926.*

Holmgren A Scandinav. J Clin. & Lab. Invest. 8 suppl. 24 1956.

Holmgren A. and *M ttson K. H Scandinav* J Clin. & Lab. Invest 6 137 1954

Holmgren A and *Persson B* Scandinav J Clin. & Lab. Invest. 12 224 1960.

Holmgren A and *Strandell, T* Acta med. scandinav 169 57 1961

Holmgren A and *St öm G* Acta med. scandinav 163 185 1959

Holmgre A Jonsson B Levander Maj Linderholm H Sjöstrand T and *Ström G* Acta med scandinav 158 413 1957

Holmgren A Jonsson B Levander Maj Linderholm H Sjöst and T and *Ström* G. Acta med. scandinav 163 239 1959

Jonsell S Acta radiol. 20 325 1939

Jonsell S and *Sjöstrand T* Acta physiol. scandinav 3 49 1941

Katz L. N Rodbard S., Friend M Rottersman W J Pharmacol. & Exper Therap. 62 1 1938

Kemp T and *Nielsen A* Statistik for medicinere Munksgaard Copenhagen, 1959

Kjellberg, S R Lönroth H and *Rudhe U* Acta radiol. 35 413 1951

Kjellberg S R. Rudhe U and *Sjöst and T* Acta radiol. 31 113 1949

Kjellberg, S R. Lön oth H Rudhe U and *Sjöstrand T* Acta med. scandinav 140 446 1951

Koelbl g H Cardiologia 17 79 1950.

LaDue J Proc. Soc. Exper Biol. Med. 46 631 1941

Lagerlöf H and *Werkö L.* Acta cardiol. 4 1 1949

Landegren J Personal communication 1964

Larsen K H and *Nielsen N A* Hospitalstid. 37 919 1936.

Larsen, K. H., Naeslund F. and *Nielsen V. A.* Am. Heart J 13 163 1937
Larsson H and *Kjellberg, S. R.* Acta radiol. 29: 159 1948.
Lepeschkin, E. Modern Electrocardiography Williams and Wilkins, Baltimore 1951
Lepeschkin E. and *Surawicz B.:* Circulation 6 378 1952.
LeVine E. B. New England J Med. 248 316, 1953
Levy R. L., Bruenn, H. G. and *Williams N. E.* Am. Heart J 19 639 1940.
Liebow I. M and *Feil, H* Am. Heart J 22 683 1941
Liljestrand G and *Nylin G* Acta physiol. scandinav 1 328 1941
Linderholm H and *Strandell T* Acta med. scandinav 162 247 1958.
Lisroth K Acta med. scandinav Suppl. 324 1957
Lloyd-Thomas, H G. Brit. Heart J 23 561 1961
Low B and Levine S A. New England J Med. 250 771 1954.
Luten, D Arch. Int. Med. 35 251 1924.
Luten, D The Clinical Use of Digitalis. Baillière Tindall & Cox, London, 1936.
McMichael, J and *Sharpey-Schafer E. P* Quart. J Med. 13 123 1944.
McMillan, T and *Bellet S* "Effects of Digitalis on the Electrocardiogram" *Walhem D Stroud* Diagnosis and treatment of cardiovascular disease. F A. Davis Company Philadelphia 1952
Michel D Deutsches Arch. Klin. Med. 201 17 1954.
Moe G. K. and *Mendez R.* Circulation 4 729 1951
Nelson, N A and *Truer M.* Am. Heart J 17 515, 1939
Nordenfelt O Acta med. scandinav Suppl. 19 1941
Nordström-Öhrberg, G Medicinska riksstämman 1958.
Nordström-Öhrberg G Medicinska riksstämman 1959
Nordström-Öhrberg, G. Unpublished observation, 1962.
Nylin, G Scandinav Arch. Physiol. 69 237 1934
Okita G. T: J Am. Geriatrics Soc. 5 163 1957
Okita, G. T Kelsey F.E., Talso P J Smith L. B. and *Geiling E. M* Circulation 7: 161 1953
Pardee H E. B. J. A. M. A. 81 186, 1923
Pick A. Circulation 15: 603 1957
Rabbe H and *Ström O.* Acta obst. et gynec. scandinav 37 312, 1958.
Rodman, T., Gorczyca, C. A and *Pastor B. H* Ann. Int. Med. 55 620 1961
Rosen I R. In *Gardberg M* Clinical Electrocardiography Hoeber Harper New York 1957
Ross J Braunwald E. and *Waldhausen J A.* J Clin. Invest. 39 937 1960.
Ross J Waldhausen J A. and *Braunwald E.* J Clin. Invest. 39: 930, 1960.
Rothlin E. and *Bircher R.* Ergeb. inn. Med. u. Kinderh. 5 457 1954.
Routier D and *Puddu, V* Arch. mal. coeur 28 800, 1935.
Raskin A. and Deckard, O Proc. Soc. Exper Biol. & Med. 68 463 1948.
Sagall, E. L. and *Wolff L.* New England J Med. 240: 276 1949
Sandberg L. Nord. med. 58 1718, 1957
Sandberg, L. Acta med. scandinav Suppl. 365 1961
Sandberg, A A., Sekeris L., Grishman A. and *Wener J* Circulation 2 921 1950.
Schröder G., Malmcrona R., Varnauskas E. and *Werkö L.* Clin. Pharmacol. Therap. 3 425 1962.
Selzer A., Hultgren, H N. Ebnother C. L., Bradley H W and *Stone A. O* Brit. Heart J 21 335 1959.
Simons G. Am. Heart J 64 401 1962.
Sjöstrand, T Acta med. scandinav Suppl. 196 687 1947
Sjöstrand T Acta physiol. scandinav 16 211 1948
Sjöstrand, T Acta med. scandinav 138 191 1950 a.
Sjöstrand T Acta med. scandinav 138 201 1950 b.
Sjöstrand T Acta physiol. scandinav 24 247 1951
Sjöstrand T Verhandl. deutsch. Gesellsch. Kreislaufforsch. 22. Tagung 143 1956

Dönhardt A Ztschr Kreislaufforsch. 40 528, 1951
Eich e L. W Taube H and *D G eff A* J Pharmacol. & Exper Therap 78 22, 1943
Erickson E. W and *Fahr G E.* Am. Heart J *29 318 1945*
Fisher R A Statistical Methods for Research Workers Oliver and Boyd Edinburgh *1950.*
Flaxman N Am. J M. Sc. 216 179 1948.
Friedberg Ch. K and *Zager A* Circulation 23 655 1961
Friedman M Bine R Byers S O and *Bland C* Circulation 2: 749 1950.
Gardberg, M Clinical Electrocardiography Harper & Brothers New York 1957
Gardberg M and *Ashman R* Arch. Int. Med. 26: 842 1943
Geiger A J Blaney L. F and *Dr ckemiller W H* Am. Heart J ?2 230 1941
Gilchrist A R Scottish M J 3 53 1958.
Gold H Fed. Proc. 3: 72 1944
Gold H Cattell M Kwit N and *K amer M* J Pharmacol. & Exper Therap. 73 212, 1941
Gold H Kwit N T Cattell McKeen and *Travell J* J A. M A. 119 928 1942.
Gold H Cattell McKeen Modell W G ei-ner T and *Guevara* R. J Pharm. & Pharmacol. 98 337 1950
Gold H Greiner T Cattell M Modell W Gluck J Marsh R Mathes S Hudso D Robertsson R Warshaw L. Otto H Kwit N and *Kramer M* Am. J Med. 13 124 1952.
Gold H., Kwit N T Otto H and *Fox T J* Clin. Invest. *18* 429 *1939*
Gold H Cattell McKeen Kwit N T and *Kramer M* J Pharmacol. & Therap. 73 212 1941
Goldberger E Unipolar Lead Electrocardiography and Vectorcardiography Lea & Febiger Philadelphia, 1953
Gremels H Arch. exper Path. u. Pharmakol. 179 360 1935
Grewin K. E. Some Supplementary leads in Clinical Electrocardiography Stockholm 1948.
Harvey R M Ferrer I Cathcart R. T and *Alexander J K* Circulation 4 366 1951
Hatcher R. A and *Weiss S* Arch. Int. Med. 29 690 1922.
Hedlund S Cardiologia 20 suppl. ad, 1952.
Helander S and *Ostman J* Svenska läk. tidn. 57 3706 1960.
Hedley O F Ann. Int. Med. 18 154 1943.
Hellström R Acta med. scandinav Suppl. 371 1961
Herrman G R Decherd G M and *McKinley W F J A. M A 18 760 1944*
Heymans J F and *Heymans C* J Pharm. & Pharmacol. 29 203 1926
Holmgren A Scandinav J Clin. & Lab. Invest. 8 suppl. 24 1956.
Holmgren A and *Mattsso K. H* Scandinav J Clin. & Lab. Invest 6 137 1954
Holmgren A. and *Pernow B.* Scandinav J Clin. & Lab. Invest. 12 224 1960
Holmgren A and *Strandell T* Acta med. scandinav 169 57 1961
Holmgren A and *St öm G* Acta med. scandinav *163 185 1959*
Holmgren A Jonsson B Levander Ma; Linderholm H Sjöstrand T and *Ström G* Acta med. scandinav 158 413 1957
Holmgren A., Jonsson B. Levander M ; Linderholm H Sjöst and T and *St öm* G. Acta med. scandinav 165 259 1959
Jonsell S Acta radiol. 20 325 *1939*
Jonsell S and *Sjöstrand T* Acta physiol. scandinav 3 49 1941
Katz L. N Rodbard S Friend M Rottersman W J Pharmacol. & Exper Therap. 62 1 1938
Kemp T and *Nielsen A* Statistik for medicinere *Munksgaard Copenhagen, 1959*
Kjellberg S R., Lönroth H and *Rudhe U* Acta radiol. 35 413 1951
Kjellberg S R. R dhe U and *Sjöstrand T* Acta radiol. 31 115 1949
Kjellberg, S R Lönroth H Rudhe U and *Sjostra d T* Acta med. scandina 140 *446 1951*
Koelbing H Cardiologia 17 79 1950.
LaDue J Proc. Soc. Exper Biol. Med. 46 631 1941
Lagerlöf H and *Werkö L.* Acta cardiol. 4 1 *1949*
Land gren J Personal communication 1964
Larse K. H and *Nielsen V A* Hospitalstid. 37 949 1936.

The aim of this study was to investigate the effect of cardioactive glucosides on the circulatory response and on the ECG reaction during muscular exercise

A total of 92 digitalizations with lanatoside C, digitoxin and digoxin was performed on 64 healthy volunteers.

After administration of cardioactive glucosides considerable reductions of heart rate at rest were observable in individual cases, though rises in some. The differences are, however not significant.

The orthostatic pulse reaction, i.e. the difference between heart rate in recumbent and standing position was considerably and highly significantly greater after administration of lanatoside C, even in small doses.

This reaction became still more marked after lanatoside C if the doses were toxic in the meaning that subjective discomfort or other symptoms appeared. No similar effect was found after digitoxin or digoxin, however not even after toxic doses.

Intravenous lanatoside C caused no significant change of heart rate during or after exercise

After oral administration of digitoxin the heart rate was rather lower during exercise at low loads, whereas there was no significant difference at higher loads. The post-exercise heart rate was unchanged before and after digitalization. Ventilation, oxygen uptake and lactic acid concentration were unchanged by digitalization

Thus digitalis did not affect the calculated physical working capacity at maximal heart rate, nor the working capacity heart rate of 170 beats/min.

It also appeared from these investigations that the degree of orthostatism in the standing position does not influence the circulatory response during sitting exercise.

The heart volume in prone position and the total amount of haemoglobin were not changed after digitalization. On the other hand the standing heart rate declined slightly both after lanatoside C and after digitoxin.

In conclusion, these studies have shown that the digitalis glucosides do not exert such a haemodynamic effect on healthy subjects as t influence the circulatory response to an exercise test at submaximal loads to a significant degree

The ECG changes in conjunction with digitalization are analysed in Part II.

The frequency of premature beats ascribable to digitalization was extremely low at rest, standing, and during and after exercise. The A V conduction was slightly prolonged after digitalization, in particular immediately after exercise. During exercise, on the other hand, there was no difference in A V time before and after digitalization.

The most common changes were observed in the ST T region and are described as 11 types.

Digitalis-induced ST T changes were recorded comparatively seldom *at rest* and were generally slight. In the standing position, on the other hand, *they* were much more common. In the total of 92 tests digitalis glucosides provoked changes in the ST T region during exercise in 89 per cent of the subjects. But immediately after exercise these changes had receded and only 39 per cent of the subjects exhibited them. Three minutes after the end of exercise the changes were somewhat accentuated,

Stewart H J and *Cohn A. E.* J Clin. Invest. 11 917 1932.

Stewart H J and *Watson R. F* Am. Heart J 15 604 1938.

Stoll A. J Pharm. & Pharmacol. 1 849 1949

Strauss H and *Katz L. N* Am. Heart J 10 546 1934

Strõm G Acta physiol. scandinav 17 440 1949

Tandowsky R. M Am. Heart J 4 472 1942.

Tandowsky R. M *Anderson N* and *Vandeventer J K* 28: 298 1944.

Tiliakos M Brit. Heart J 15 95 1953

Wahlund H Acta med. scandinav Suppl. 21. 1948.

Walton R. P., Leary J S and *Jones H P* J Pharm. & Pharmacol. 98 346 1950.

Wedd A. M and *Blair H A* J Pharmacol. & Exper Therap 90 211 1947

Wégria R. Pharmacol. Rev 3 197 1951

Werkö L. Eliasch H Thomasson B and *Varnauskas E.* Cardiologia 33 161 1958.

Wiggers C J and *Stimson B.* J Pharm. & Pharmacol. 30 251 1927

Wiklander O Acta chir scandinav Suppl. 208 1956

Williams H Jr Zohman L. R. and *Ratner A C* J Appl. Physiol. 13 417 1958.

Withering, W An Account of the Foxglove and Some of its Medical Uses with Practical Remarks on Dropsy and other Diseases. G. G J and J Robinson, London, 1785 Reprinted in Medical Classics, 2 305 1937

Wood P Diseases of the heart and circulation. Eyre and Spottiswoode, London 1956

Woodbury L. A. and *Hecht H H* Circulation 6: 172, 1952.

Wright S E. The metabolism of cardiac glucosides. Charles C. Thomas Publisher 1960.

Yuletic I and *Ivancic R.* Arch. mal. coeur 45: 853 1952.

Yu P N G., Lovejoy F W Hulfish B. Howell M M Joos H A Tenney S M Haroutunian L. M and *Evans H W* Am. J M. Sc. 224 146 1952.

Zwillinger L. Med. Klin. 31 977 1935

Åstrand Irma Acta physiol. scandinav Suppl. 169 1960.

Åstrand P O Experimental studies of physical working capacity in relation to sex and age. Munksgaard Copenhagen, 1952.

ACKNOWLEDGEMENTS

I wish to express my sincere thanks to Associate Professor Bengt Pernow head of the Department of Clinical Physiology at Serafimerlasarettet, for his stimulating advice and interest and his very generous support during the course of this work. My thanks are due also to Professor Gunnar Biörck for valuable discussions and for his profitable advice concerning the preparation of the manuscript.

I also wish to thank Professor Gunnar Ström, Uppsala, during whose time as head of the Department of Clinical Physiology at Serafimerlasarettet this work was started, for his encouragement during the entire course of the study and for his advice and constructive criticism.

My special thanks are due to Nurse Ingrid Ollén for her assistance, to Mrs. Agnes Rotschild and Miss Berith Hultenberg for help with the laboratory work, to Mr Torkel Gårding who did most of the statistical analysis, and to Mr John Hogg for his translation into English. Also to Mrs. Birgit Hambring who, throughout the whole study has given me skilled, valuable and always encouraging assistance in typing.

The investigation was supported by grants from the Swedish National Association against Heart and Chest Diseases.

and 48 per cent of the subjects nown showed ST T changes.

The changes during the post-exercise period were not more pronounced than during exercise.

If the ST T region was unchanged during exercise, no ST T changes occurred either in digitalized subjects after exercise.

There was no difference as regards the effect of lanatoside C and digitoxin on the ST T region. Nor does this study show that digoxin has a different effect on the ECG from that of the two other glucosides.

One may thus say in conclusion that digitalis glucosides may cause several types of ST T changes, and that in the exercise ECG some of these changes are virtually undistinguishable from those reported as typical of coronary insufficiency The appearance of ST T changes already in the standing position, and the quick restitution of the digitalized subjects after exercise, however may be of some assistance for the differential diagnosis. But as shown in this study there are occasions when the ST T region is not changed in the standing position and does not become normalized after the end of exercise.

It must therefore be emphasized that, to avoid false positive responses to exercise tests, it is important that, in the case of a compound with a prolonged effect such as digitoxin, a sufficiently long period (at least six weeks) should have elapsed since the administration if the intention is to assess from the ECG reaction whether coronary insufficiency is present or not.

In this investigation the effect of digitalis glucosides was studied on subjects with healthy hearts. It is conceivable that in cases of at least badly diseased hearts the effect may be different.

ACTA MEDICA SCANDINAVICA

SUPPLEMENTUM 419

A STUDY OF BRONCHIAL ASTHMA

with special reference to its long term consequences for respiratory and circulatory function and socio-medical condition

By

LARS IRNELL

Accompanies Vol. 176

UPPSALA 1964

ACTA MEDICA SCANDINAVICA

has been published since 1919 as a continuation of Nordiskt Medicinskt Arkiv founded in 1869 by Axel Key The first volume of Acta Medica Scandinavica *is therefore numbered LII (52)*

The chief editors have been Axel Key 1869—1900 C. G Santesson 1901—1915 I Holmgren 1916—1957 *and Birger Strandell 1958 to date.*

Acta Medica Scandinavica publishes original research papers in the field of internal medicine Priority will be given *to authors from countries which* are represented on the editorial board. Contributors from those countries should submit their papers to a representative of the country in question Contributors from other countries should send their papers to the Editor at the address below

Submission of a manuscript for publication will be held to imply that the paper has not been published elsewhere and that if accepted, it will not be republished in any other journal in the same or similar form without the Editor s written consent

Papers will be published in English French or German at the authors choice followed by a summary in English

The manuscripts shall be in final form as submitted They shall be type written with double spacing and broad left hand margin.

If a paper exceeds 16 printed pages the cost for the excess pages shall be borne by the author No paper shall exceed 24 printed pages.

Subscription

The annual subscription *to the journal covering two volumes*, each of 6 numbers, is 140 Sw crowns or U S $ 27 25 *including postage* in the Scandi*navian countries and in Holland 120 Sw* crowns

Address for subscriptions and all communications
ACTA MEDICA SCANDINAVICA
P O Box 2052 Stockholm 2

Requests for duplicate copies of numbers that have gone astray can only be considered if made within a *fortnight of the receipt of the* following number

From the University Hospital, Uppsala, Sweden

Department of Internal Medicine
Head: Professor E. A. K. Upmark, M.D.

Department of Clinical Physiology
Head: Professor G. Ström, M.D.

Department of Social Medicine
Head: Professor R. Berfenstam, M.D.

A STUDY OF BRONCHIAL ASTHMA

with special reference to its long term consequences for respiratory and circulatory function and socio-medical condition

By

LARS IRNELL

Statistical adviser: Gunnar Eklund, Ph. D.

UPPSALA 1964

ACTA MEDICA SCANDINAVICA

has been published since 1919 as a continuation of Nordiskt Medicinskt Arkiv founded in 1869 by Axel Key The *first volume of Acta Medica Scandinavica* is therefore numbered LII (52)

The chief editors have been Axel Key 1869—1900 C. G Santesson 1901—1915 I Holmgren 1916—1957 and Birger Strandell 1958 to date.

Acta Medica Scandinavica publishes original research papers in the field of internal medicine Priority will be given to authors from countries which are represented on the editorial board. Contributors from those countries should *submit their papers to a representative of the country in question.* Contributors from other countries should send their papers to the Editor at the address below

Submission of a manuscript for publication will be held to imply that the paper has not been published elsewhere and that if accepted, it will not be republished in any other journal in the same or similar form without the Editor s written consent

Papers will be published in English French or German at the authors choice followed by a summary in English

The manuscripts shall be in final form as submitted They shall be type written with double spacing and broad left hand margin

If a paper exceeds 16 printed pages the cost for the excess pages shall be borne by the author No paper shall exceed 24 printed pages

Subscription

The annual subscription to the journal covering two volumes, each of 6 numbers, is 140 Sw crowns or U.S $ *27 25 including postage* in the Scandinavian countries and in *Holland 120 Sw crowns*

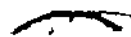
Address for subscriptions and all communications
ACTA MEDICA SCANDINAVICA
P O Box 2052 Stockholm 2

Requests for duplicate copies of numbers that have gone astray can only be considered if made within a fortnight of the receipt of the following number

CONTENTS

Also published as
SUPPLEMENT
of ACTA MEDICA SCANDINAVICA

Translated by Maud Marsden



CONTENTS

Also published as
SUPPLEMENT
of ACTA MEDICA SCANDINAVICA

Translated by Maud Marsden

I INTRODUCTION AND AIM OF THE INVESTIGATION

Those treating patients for bronchial asthma, often in a serious condition, may ask How does a disease such as this affect the individual in the long view? Many of the patients appear in hospital time and time again, often in an apparently serious condition They may become 'regular customers both in the out patients department and on the wards, and from their histories of frequent, more or less pronounced symptoms, it may be assumed that for many of them the disease has indeed influenced their lives.

Others belong to the group of patients who, although they may also seek hospital treatment on one or more occasions with severe asthma, are then seldom or never seen there again. Of their subsequent progress little is known after their few hospital visits there is often no further information either on their ward case records or their out patient cards. Have they recovered? Or has the asthma changed in character so that the acute attacks necessitating hospital treatment have subsided? Have special efforts on the part of the patients doctor or community produced a change for the better

Questions of this kind are of especial interest to the clinician, and it was therefore considered justifiable to study this matter further

---

It is difficult to obtain adequate information on the prognosis in adult patients with bronchial asthma, for several reasons. The main difficulty is caused by the confusion in the literature as to the definition of the terms bronchial asthma chronic bronchitis and pulmonary emphysema. Each of these terms sometimes appear to be used by different clinicians to describe the condition in one and the same patient.

The large spontaneous variations and often very irregular course of this disease have probably to a great extent discouraged detailed analyses of its influence on adult persons. The investigations described in the literature have usually comprised small series of cases, and as a rule attention has been paid to only one or a small number of those factors which when combined contribute towards a summarizing assessment of the prognosis of the disease. In the different investigations reported attempt has often been made to correlate the prognosis of the

Uppsala 1964
Appelbergs Boktryckeri AB

II DEFINITIONS

Considerable attempt has been made during recent years to produce exact clinical definitions and classifications of bronchial asthma, chronic bronchitis and pulmonary emphysema—summarized in the term chronic obstructive pulmonary diseases. Different authors have tended to define these conditions in different ways. The lack of generally accepted definitions and classifications has obviously delayed clinical research in this field, and has also decreased the possibility of utilizing to its full extent the experience gained, of evaluating results and making comparative studies. It is clear that further knowledge of these diseases is required for the establishment of more generally accepted definitions

As pointed out by the American Thoracic Society (ATS) Committee on Diagnostic Standards for Nontuberculous Respiratory Diseases (1962) a disease may be defined according to its etiology the characteristic morphological changes which it causes, the functional disturbances it produces or its clinical manifestations In chronic bronchitis and bronchial asthma there appears to be no individual manifestation or combination of criteria which permits an exact definition. The ATS further states that the definitions of these conditions are therefore based essentially on the description of clinical manifestations which in themselves are not pathognomic. In pulmonary emphysema on the other hand, the anatomical changes are sufficiently specific to allow with the use of anatomical terminology a definition of this condition.

In the following, definitions of bronchial asthma, chronic bronchitis and pulmonary emphysema, as used in the present investigation, will be given. The recommendations published by the ATS were followed to a large extent. The descriptions in quotation marks are cited from these recommendations.

Chronic bronchitis Chronic bronchitis is a clinical disorder characterized by excessive mucous secretion in the bronchial tree. It is manifested by chronic or recurrent productive cough.

The expectoration should not be due to localized broncho-pulmonary disease or cardiac disorders. I have defined the words chronic or recurrent as present on practically all days during at least three months in each of more than two successive years.

Bronchial asthma Asthma is a

asthma with certain forms of therapy or certain types of the disease, less attention being paid to general assessments of functional sequelae.

It may be assumed that during the early stages of the disease the bronchial obstruction and its sequelae are only temporary. On the other hand it may be expected that repeated attacks or prolonged periods of difficulty in expiration may lead to irreversible changes especially pulmonary but also cardiac. Similarly socio-medical functional impairment might also be expected. *The aim of the present investigation was to describe the clinical course of the disease in a series of adult patients with bronchial asthma of varying duration and severity selected according to certain norms for the purpose of studying—in relation to the estimated severity and course—the long-term effect of the disease both on the respiratory and circulatory functions and on the social situation of the patients.*

The effect of the disease on the respiratory and circulatory functions was assessed by studying the ventilation capacity lung volumes and physical work capacity and also hemodynamic data obtained by means of right heart catheterization. The results obtained were compared with corresponding data from control materials and were also correlated to the estimated degree of severity of the disease.

The effect of the disease on social adaptation was studied by assessing the status of the patients in this series as compared with control materials, using certain indicators. The social situation of these patients was studied for the year 1960 and comparisons were made with the situation for the corresponding total population as indicated by the housing and population census for that year and other available control materials. Among the indicators studied, the main items were family data general education, changes of residence, adaptability with regard to life at work, income, and housing conditions.

II DEFINITIONS

Considerable attempt has been made during recent years to produce exact clinical definitions and classifications of bronchial asthma, chronic bronchitis and pulmonary emphysema—summarized in the term chronic obstructive pulmonary diseases. Different authors ha e tended to define these conditions in different ways. The lack of generally accepted definitions and classifications has obviously delayed clinical research in this field, and has also decreased the possibility of utilizing to its full extent the experience gained, of evaluating results and making comparative studies. It is clear that further knowledge of these diseases is required for the establishment of more generally accepted definitions.

As pointed out by the American Thoracic Society (ATS) Committee on Diagnostic Standards for Nontuberculous Respiratory Diseases (1962) a disease may be defined according to its etiology the characteristic morphological changes which it causes, the functional disturbances it produces or it clinical manifestations. In chronic bronchitis and bronchial asthma there ppears to be no individual manifestation or combination of criteria which permits an exact definition. The ATS further states that the definitions of these conditions are therefore based essentially on the description of clinical manifestations which in themselves are not pathognomic. In pulmonary emphysema, on the other hand, the anatomical changes are sufficiently specific to allow with the use of anatomical terminology a definition of this condition.

In the following, definitions of bronchial asthma, chronic bronchitis and pulmonary emphysema as used in the present investigation, will be given. The recommendations published by the ATS were followed to a large extent. The descriptions in quotation marks are cited from these recommendations.

Chronic bronchitis Chronic bronchitis is a clinical disorder characterized by excessive mucous secretion in the bronchial tree. It is manifested by chronic or recurrent productive cough.

The expectoration should not be due to localized broncho-pulmonary disease or cardiac disorders. I have defined the words chronic" or recurrent as present on practically all days during at least three months in each of more than two successive years.

Bronchial asthma. Asthma is a

disease characterized by an increased responsiveness of the trachea and bronchi to various stimuli and manifested by a widespread narrowing of the airways that changes in severity either spontaneously or as a result of therapy

In the present study the term bronchial asthma is not used if the criteria of chronic or recurrent cough are considered to be fulfilled in accordance with the conditions stated in the definition of chronic bronchitis In agreement with the ATS I consider that "the term asthma is not appropriate for the bronchial narrowing which results solely from destructive diseases of the lung e. g pulmonary emphysema or from cardiovascular disorders

The characteristics of the clinical manifestations of bronchial asthma found in the present study are in concordance with the *description made by the ATS* where bronchial asthma is said to be clinically characterized by episodes of dyspnea, cough and wheezing These episodes may be transitory and subside within one or two days, or they may be prolonged. Some episodes may be extremely severe and result in death. During these episode physical examination may reveal prolongation of expiration, an over expanded thorax and hyperresonance to percussion On auscultation, high pitched wheezing sounds are heard throughout the entire thorax they are audible both in inspiration and expiration but are more prominent in expiration.

Characteristic of the disease is its intermittent course with symptom periods of varying lengths between more or less symptom free intervals. There is usually an allergic background, but this cannot always be shown.

Pulmonary emphysema. Emphysema is an anatomic alteration of the lung characterized by abnormal enlargement of the air spaces distal to the terminal non respiratory bronchiole accompanied by destructive changes of the alveolar walls.

III SURVEY OF LITERATURE

Owing to the lack of generally ac cepted definitions of chronic obstruc tive pulmonary diseases, it is difficult in studying the literature to form an idea of the sequelae of bronchial asthma. When this condition is de fined, which is fairly seldom, the definitions vary often depending on the disciplin or country in which the author is active. Clear delimitation from other chronic obstructive lung diseases is not often made.

On tudying the literature attempt was made to obtain data on the fol lowing

1 Results of clinical follow up ex aminations of patients with chronic obstructive pulmonary disease.
2 Result of studies aimed at find ing out whether the cardio-respi ratory functional changes in bronchial asthma differ from those in other chronic obstructive pulmonary diseases.

   Special ttention was paid to the effect on

   respiratory function, which can be assesed by means of determina tions of the lung volume and entilation capacity (2 a)

   and circulatory function, which can be assesed by means of flow and pressure determinations made during right heart catheterization (2 b)
3 Socio-medical aspects of patients with chronic obstructive pulmonary disease (3 a)

No reports of systematic investi gations treating essential indicators of the sequelae of such diseases from the point of view of social adaptation were found. It was therefore considered justified, for the purpose of discussing the social consequences of this disease in comparison with other chronic morbid conditions, to make a brief complementary review of the lite rature concerning socio-medical sequelae of chronic diseases in general concentrating on Swedish investigations (3 b)

Clinical follow-up studies

Ogilvie (1962) reported study on 1000 unselected patients with bronchial asthma who were f llowed up for an erage period of 11 years. Bronchial sthma wa defined condition haracterized by ecurrent bout of wheezing, mainly or entirely ex piratory with an a sociated ariable degree of difficulty in breathing and f expiratory obstruction If the sthma had symptom free inter l it w described as intermit tent nd if wheezing nd dyspnoea per sisted it wa called continuou Chronic

bronchitis was defined as a condition characterized by cough and flegm persistent throughout the winter or throughout the year with a minimum duration of two years the absence of other diseases of the lungs is implicit It may be stressed that many of the 1000 patients in this series had or had had chronic bronchitis besides their asthma.

This author found that the prognosis in the intermittent form of asthma was more favourable than in the continuous type. The outlook was also found to be more favourable in thos cases where the disease was manifested before the age of 16 years The risk that the asthma would gradually become complicated by bronchitis was greater for those with the continuous type. At the follow up examinations the general condition of the asthmatic patients with bronchitis appeared to be much poorer than that of the patients without this complication. The author claimed to have shown that treatment with specific hyposensitization had no noteworthy effect on the long term prognosis.

Reid and Fairbairn (1958) reviewed the ickness records of 517 postmen who had retired prematurely because of chronic bronchitis, and noted their death rate and causes of death after intervals varying from three to seven years. The ratio of observed to expected deaths was 4.5 1

Med ei and Oswald (1962) reported a five-year follow up study of 312 bron hitic pa tients. Approximately 75 /₀ of the patients were men of ages 45–64 years. The criteria for inclusion in the study were co gh and sputum, not necessarily continuous for at least a year which could not be attributed to any other important primary or precipi tating disease of th respiratory cardiovascular or other ystems The ratio of observed to expected deaths f the males was 4.2 1 this declined with increasing age The authors stated th t this ratio sh wed good agreem nt with that reported earlier by Reid and Fairbairn (1958) It w pointed out, further that other results from these two investigations also showed strikingly high degree of concordance For exampl in 63 / of th patients who died during th observati n period, the cause of death was respiratory insufficiency The corresponding figure in the investigation of Reid and Fairbairn was 58 %. In both studies circulatory insufficiency was the cause of death in 20 % of the cases. In Medvei and Oswald's material 96 patients (31.5 %) died during the five year period. It was stated that in all of the 5 patients in whom the cause of death was respiratory insufficiency chronic bronchitis and pulmonary emphysema appeared to be the underlying cause.

With regard to the subsequent development of asthma manifested at an early age, the pediatric literature contains mu h information, and reference may be made to the reviews published by Kraepelin (1959 Aas (1963) and Rysing (1963)

Respiratory function

As pointed out by Gaensler (1962) until the end of the 1950 s there was practically no sub-division of the chronic obstructive pulmonary dis eases in the reports of physiological data from these patients. It was at this time that more intensive efforts to define and differentiate between bron chial asthma, chronic bronchitis and pulmonary emphysaema were begun

Three monographs (Stuart Harris and Hanley 1957 Ogilvie and Newell 1957 Oswald 1958) treating chronic bronchitis and to some e tent the functional impairment ascribed to thi disease, were published at the end of the 1950's.

The problem of definition and differe tia ti n in thi disea e group, however is not yet completely sol ed. Som light i shed on th question by the fact that Benzetti, East man, Auchincloss, Gilbert Eich and D tton (1958) who carried out physiological studies on patient with chronic bronchitis, limi nated some of these from the final result because their residual volume con tit ted more than 35 % of the total lung capacity a classification that must be regarded a highly arbitrary Chronic bronchiti wa d

fined condition i which t lea t 10 weeks f coughing occurred each day for t least six months, provided that there were no signs of othe broncho-pulmonary disease This definition is also obviously bitrary

Fletcher Hugh-Jones, M Nicol and Pride (1963) studied the occurrence of pulmonary emphysem in chronic bronchitis. These authors found that many of the clinical nd physiological changes that really regarded as indicating the presence f pulmonary emphysema, only do thi in the func tional sense i e. in the sense of hyper inflation. These changes cannot be regarded as evidence of the presence of destructiv emphysema. Th authors recommend that the word emphysema should be reserved for cases where obvious functional and roentgenological signs of l eolar tissu destruction re found.

The above investigations are examples of those aimed at studying one of the group of obstructive pulmonary diseases, in these cases chronic bronchitis Only exceptionally have reports been made of investigations on patients with bronchial asthma who could all or practically all be considered free from chronic bronchitis.

One of these exceptions is the lung function study carried out by Herschfus, Bresnick nd Segal (1953) on 43 asthma patient of ages 18–72 years, in symptom-free inter l or periods when the symptoms wer least pronounced The vital capacity was found to vary between 61 nd 158 / and the ma imum oluntary entilation between 11 nd 130 in both cases f the predicted normal alues. The mean total lung capacity wa 123.8 of the predicted normal value, nd the residual quotient aried between 35 and 67 % with mea valu of 47 The mean ma imum pirator olume per sec ond as 3.9 litres

In several reports, although the title indicates that the results concern only patients with bronchial asthma mention may be found in the text that the material consists of a mixture of patients with asthma chronic bronchitis and emphysema If the functional changes differ in the different types of chronic obstructive pulmonary diseases, an intermixture such as this must obviously result in misleading reports. Because of the confusion in the diagnoses of patients in the earlier studies, it is often difficult when attempting, for example to assess the long term effects of bronchial asthma as such to interpret the results and gain any direct information.

I the ea lier literature there re many eport f results from determinations f the lung olume and/or entilation capa ity in emphysema, for example Lundsgaard nd Schierbeck (1922) Hurtado, Kaltreider Fray Brooks nd McCann (1934) Baldwin (1946) Baldwin, Cournand nd Dickinson (1949) Bates, Knott nd Christie (1956) Beale, Fowler and Comroe (1952) Pa k nd Lee (1961) h ip (1961) Kahana, Aronovit h nd Plare (1963)

Also of importance in assessing the results from the different investigations is detailed knowledge of the apparatus used in the dynamic lung func tion tests the results depend among other things on the amount of resist ance to respiration provided by the apparatus. The differences in the meth ods used, together with the frequent lack of definition of the disease con dition described have constituted great barriers to the analysis and assessment of the studies mentioned. These investigations thus provide no definite guidance with regard to the problems of the present study

bronchitis was defined as a *condition characterized* by cough and flegm persistent throughout the winter or throughout the year with a *minimum duration of two years* the absence of other diseases of the lungs is implicit It may be stressed that many of *the 1000 patients in this series* had or had had chronic bronchitis besides their asthma.

This author found that the prognosis in the intermittent form of asthma was more favourable than in the continuous *type. The* outlook was also found to be more favourable in those cases where the disease was *manifested before the age of 16 years.* The risk that the asthma would gradually become complicated by bronchitis was greater for *those with the continuous* type. *At the* follow up examinations the general condition of the asthmatic patients with bronchitis appeared to be much poorer than that of the patients without this complication. The author claimed to have shown that treatment with specific hyposensitization had no note*worthy effect on the long term prognosis.*

Reid and Fairbairn (1958) reviewed the sickness records of 517 postmen who had retired prematurely *because of chronic bron*chitis, and noted their death rate and causes of death after intervals varying from three to seven years. The ratio of observed to expected deaths was 4.5 1

Medvei and Oswald (1962) reported a five-year follow up study of 312 bronchitic pa*tients. Approximately 75 % of the* patients were men of ages 45-64 years. The criteria for inclusion in the study were cough and sputum, *not necessarily continuous* for at least a year which could not be attributed to any other important primary or precipitating *disease of the respiratory cardio*vascular or ther systems The ratio of observed to expected deaths f r the males wa *4.3 1 this declined with increasing age.* The authors stated that this ratio showed good agreem nt with that reported earlier by Reid and Fairbairn (1958) *It was pointed* out, further that other results from these two investigations also showed a strikingly high degree of concordance *For example in* 63 / of the patients who died during the bserv tion period, the ca se of death was respirat ry insuffici ncy *The corresponding figure* in the investigation of Reid and Fairbairn was 58 %. In both *studies circulatory* insufficiency was the cause of death in 20 % of the cases. In Medvei and Oswald's material 90 patients *(31.5) died during the* five-year period. It was stated that in all of the 57 patients in whom the cause of death was *respiratory insufficiency* chronic bronchitis and pulmonary emphysema appeared to be the underlying cause.

With regard to the ubsequent development of asthma manifested at an early age. the *pediatric literature contains much in*formation, and reference may be made to the reviews published by Kraepelin (1950) *Aas (1963)* and Ryssing (1963)

Respiratory function

As pointed out by Gaensler (1962) until the end of the 1950 s there was practically no sub division of the chronic obstructive pulmonary dis*eases in the reports of physiological data from these patients* It was at this time that more intensive efforts to define and differentiate between bronchial asthma, chronic bronchitis and pulmonary emphysema were begun.

Three monographs (Stuart Harris and Hanley 1957 Ogilvie and Newell 1957 Oswald 1958) treating chronic bronchitis and to some extent the functional impairment ascribed to this disease, were published at the end of the 1950 s.

Th problem of definition and differentiation in this disease gro p, however is not *yet completely* solved. Some light is shed n the question by the fact that Renzetti, Eastman, Auchincloss, Gilbert, Eich nd Dutton *(1958)* wh *ca ried o t physi* logical studies on patients with chronic bronchitis, eliminated some of these from the *final* result because *their* residual volume constituted more than 35 % *of the total* lung capacity a classificati n th t m st *be regarded* a highl arbitrary Chro ic bronchitis was d

condition he considered later however that owing to the una liability f adequat data thi had not been particularly successful. The material was collected with the as sistance of number of general practitioners in different parts f Norway. A questionnaire w sent to those asthma patients of whom the thor had received information from these practitioners. Claussen states that the material con isted of just over 1500 patient The thor giv the percentage distribution of diff rent occupations of the male patient living in certain ru l districts, and com pared them with the occurrence of the respectiv occupations among the entire mal rural district population in Nor y. The handicraft trades nd f rming and forestry work ere well represented among the sthma patients, while there w nder representation w th regard to ind try. Claussen found that 7.3 of th men in the material ere complete! unfit for work due to thei asthma The erage age of these men wa 53 ears, and the average duration of the sthma 18 years. 4.5 / f the women wer completely unfit for work, their average ge was 44 ears and th erage duration f the sthma 16 ears I the maly of these figure only men and women of ges 18–70 ears were included. Of these who were completely incapacit ted, the majority had had their first onset of sthma t an adult age The duration of the asthma I these patient not longer than in the whole material The total number of com pletely disabled sthmatics of ges 18–70 ears Norw wa estimated t be at lea t 700 A comparison t as mentioned that ac ording to Gram (1931) there wer 613 sthmatics in Denmark in 1931 who were registered completely disabled by reason of th disease

With regard t the non-disabled, in the majority of cases the capacity for work w more or les reduced This was evidenced to great part by changes in occupation or re duction f orking hours. Information on the number of working da lost was only btained for those nswered persons, who ere able to state the length of time for which they had recei ed sickness benefit because of thma. For the men, 220 L together there wa n average f 20 day I 1945

No ttempt t defi ition of the disease nd differentiation from chronic bronchitis was made. An investigation of this type based on information from several practising doc tor t different district seriously present great difficulties, nd an ssessment f the exact umber of disabled broncho-a thmatic patient i hardly possible. The term dis abled wa not defined the patients them sel es judged whether or not they were completely fit for work. The comparison bet een the di tribution of the diff rent oc cupations for the sthmatics nd for the whole rural population of Norway had t be made with great care, since the author states, the population in the district in estigated did not hav the sam occupatio nal distribution that i the tot l rural di tricts.

I an article entitled Social Importance of Allergic Diseases Williams (1951) re ported his study of the thma mortality in the d fferent social groups in England and W les t the beginning of the 1930's. The mal rial was divided int 5 social groups. Group I and to some extent group II, which were the highest, had a lower mortality tha the other social groups The thor f rther ted investi gation on the numbe of ick ness days per 10,000 individual with sthma nd other allergic diseases, respectively in the population f England nd W les. Som hospit l stat stics on the incidence of asthma diagnosis were also given — both bsolut figures and stati in relation t other diagnoses In 2 2 f approximatel 210,000 patient discharged f om bout 20 large hospital in E gland, Scotland and Wales thma was the main diagnosis. Will iams did not define sthma, and the term probably covered all chronic obstructive pul monary diseases

Ingbe 1949 found probably increased asthma morbidity among the female pau pers in Stockholm, while there was no cor responding increase for the men. The author ssumed that thi sex difference was due to the fact that sthma, apart from acute cases, as rule only causes slight disability nd that moderat degree of disablement

Circulatory function

A relatively large number of reports have been published on the effect of chronic obstructive pulmonary disease on circulatory function As a rule these have concerned studies on series of cases with relatively advanced pulmonary emphysema Patients with chronic bronchitis or bronchial asthma have usually been represented in the various investigations but in most studies patients with the diagnosis of pulmonary emphysema predominate. It is often clear to the reader however that in many of these patients the primary disease was chronic bronchitis bronchial asthma or a clinical condition characterized by symptoms typical of both these diseases No systematic investigations appear to have been made with the aim of following up patients who during a given period were admitted to hospital on one or more occasions for bronchial asthma—with no chronic bronchitis Neither do any studies appear to have been made for the purpose of correlating several different case-history data concerning the estimated degree of severity of the disease, with the findings made in hemodynamic studies

Wade a d Bi h p (1002) compared the results from a number of in estigati on th resting cardiac outp t in chronic bronchiti and pulmonary emphysem It was pointed ut that the designati n emphysema ref rred to patients who had exhibited widely aried clinical pictures with orrespondingly varied patterns of disturbed cardio-respiratory function. The comparisons w re based on 216 observations in 201 patients with mphysema. In 58 instances the pulmonary arterial pressure wa n rmal, the mean pressure not exceeding 22 mm Hg nd in these patients the mean cardiac index was 3.37 l/min m² — In a further 101 observations pulmonary hypertension was present but the patient was not in congestive failure. The mean cardiac index in these patients was 3.43 l/min m — Finally there were 57 observations on patients with pulmonary hypertension who were in congestive failure In these patients the mean cardiac index was 3.50 l/min m² — The presence or absence of congestive failure was not always learly stated but it was regarded as present if the mean right atrial pressure was greater than 7 mm Hg — The three groups of patients did not differ significantly in the level of their cardiac output which was in each instance essentially the same as that in normal subjects.

Some auth rs may be mentioned here who have previously studied hemodynamic function in pulmonary emphysema Borden, Wilson Ebert and Wells (1050) Mounsey Ritzmann Selverstone, Briscoe and McLemor (1032) Harvey Ferrer Ri hards and Cournand (1951) Fowler Westcott Scott and Hess (1952) Yu Lovejoy Joos, Ny and McCann (1053) Whitaker (1054) and Scherre (1061)

*It is evident in several of these in*vestigations that many patients with *pulmonary diseases belonging to the* chronic obstructive group exhibit residual impairment to circulatory function, that is not rarely incapacitating As in the case of the lung volume and ventilation capacity studies, however the conclusions drawn from these reports give no definite guidance with regard to the present problems

Socio-medical aspects of chronic obstructive lung diseases

Claussen (1948) in an article entitled Asthma in Norway aimed at st dying the incidence of the disease f that co try nd its social significance a an incapa itating

increasing age. 2 0 % of the population be tween 50 and 60 years of age were denoted disabled. The women were disabled to pproximately the same extent as the men, but their disablement ppeared to begin t somewhat younger age. 1.7 of the popu lation studied by Bentsen wer considered t be the asthma-bronchitis-emphysema syndrome. But f these only 28.7 / were assigned to the sthma group, and of this percentage number (the percentage was not tated) had bronchitis, the duration of which had exceeded 3 months.

It would be been of great dvantage if Bentsen, in discussing the disablement aspect of th material, had ttempted t dif ferential between asthma, chronic bronchitis and emphysema. With the lassifications presented in the material this ppears to ha been possible. No conclusions can be drawn with regard to the group of bronchial asthma patients, from th result given, since this group pparently constitutes less than 1/4 of the material.

Mork (1962) compared the morbidity nd mortality in respiratory diseases in England & Wales and Norway. The author reviewed the studies in which epidemiological aspects of espiratory diseases were treated. H also investigated, on the basis of information obtained from several countries, the correlation between the mortality and number of rables such air pollution, population density degree of urbanization and industrialization, occupation and different indices of the tandard of living. N significant relationship was found, however. The results of the tud of the morbidity data from England & W les and Norway indicated that there was some difference in morbidity between these countries with regard to bronch itis and pneumonia, but th difference w smaller than that found for the mor tality. The uthor regarded this as an indication that the course of the non-specific respiratory diseases in England and Wales was more serious and that the mortality was higher.

This thor studied male transport workers of ges 40-59 years, in Bergen (Norway) nd also in London, nd compared the prevalence of respiratory symptoms, the ventilation capacity nd smoking habits. The London material showed higher prevalence of respiratory symptoms, and t the same time the ventilation capacity was lower. It was not considered that th difference shown between the tw series could be explained by difference in nthropometric alues or in socio-economic factors. The dissimilarities in t bacco consumption wer assumed to be of significance in the differences in prevalence of a minor espiratory syndrome haracterized by cough nd the production f phlegm. The difference in the incidence of more serious symptom ("f tal chronic cardio-respiratory disease") and in the reduction of entilation capacity was assumed t be due t the difference in exposur to tmospheric pollution between the two series.

Walkup nd Connelly (1963) report the incidence of chronic respiratory diseases in th U.S.A., according t the N tional Health Survey of 1960. Persons with asthma-hay fever are stated to be pproximat ly six times as common as those with chronic bronchitis but sthma wa the reason for only about half many days of bed disability per year per person (2.8 and 5.3 days, respecti ly). The thors report further the percentage distribution of deaths from chronic respiratory diseases in 1959. Asthma constituted 0.3 of all causes f death. The corresponding figure for bronchitis and emphysema were 0.2 and 0.5 % respectively.

The information given can be said to illustrat th relatively great significance ttached t asthma in the U.S.A. as compared with chronic bronchitis. T certain extent the conditions in England ppear to be the opposite which has been illustrated by several thors, including Mork (1962). E en if there is true difference between the two countries, it is probable that the data given will be highly influenced by dissimilarities in the diagnostic criteria for these condition.

For discussion of bronchial sthma and other chronic obstructi pulmonary diseases with regard to occupational spects, re ference may be made to the reviews published by Hamperdinck (1962) and Koelsl g (1963).

in the men need not result in unemployment, if work is available. For women on the other hand, the author considered it possible that a handicap such as this could have severe consequences.

In an article entitled Socio-medical aspects of bronchial asthma in Israel Groen and Lieber (1960) gave some statistical information from hospitals. In a so-called one day survey" comprising the patients of all *hospitals in Israel (with the exception of* tuberculosis and mental hospitals) it was *found that the bronchial asthma patients* constituted 2.2 % of those with chronic *diseases.* When the patients with acute and chronic bronchitis, bronchiectases and cor *pulmonale (called related disorders")* were included, the figure rose to 3.5 %. The num*ber of hospital admissions for bronchial* asthma during the period 1949–1955 constituted *0.5–0.8 % of the total If the related* disorders were included this figure rose *to 1.5 %. The mean period of hospitalization* during the period 1953–1955 for all diseases combined, was 21 days *for men and 15 for* women. For bronchial asthma the corresponding period was 15 and 11 *days,* respectively The number of hospital admissions for bronchial asthma was greater than for malignant gastric tumours and approximately the same as for diabetes mellitus.

Because of the nature of the investigation —the inform tion was obtained from case records and archives of the Israeli hospitals —it was not possible to define the bronchial asthma precisely and to distinguish it from chronic bronchitis, which was the main diagn is for about half of the patients who were assigned to the group of related dis orders In the main, however the information provided by this study ppears to contribute much to the elucidation of the prob- l ms concerning bronchial asthma nd th so-called related disorders.

Hollman (1961) in his article Das Bronchialasthma *als soziale Krankheit* reviewed some eports made by different authors both *in West Germany* and England on th number of working days lost owing to chronic diseases of the respirat ry tract The number f members of the West German Health Insurance Servic who were unfit f r work because of asthma was stated to be 17 for the year 1937 27 for 1952 and 33 for 1956, per 10 000 insured. The number of days for which sickness benefit was received because of asthma was 320 000 for 1957 for the 25 million members.

This information was obtained from the official statistics of the Health Insurance Service, but from a scientific point of view it must be regarded with reservation since the definition and diagnosis of these respiratory diseases probably varied considerably among the doctors making the reports upon which the analysis was based. No attempt was made in this article to define the dif ferent conditions. Hollman assumed that the broncho asthmatic diseases, mainly because of their morbidity would be assigned in the near future to the group of the most common diseases.

Susan Meadows (1961) studied social class migration and chronic bronchitis in male *hospital patients in the London area. The* author chose a hospital material for her *tudy on the assumption that the bronchitis* in these patients would be of a relatively *evere type* It was found that during the five years immediately preceding the investigation there had been an increase in the number of bronchitic patients in the lower social classes. No corresponding increase was found in a control material. The author believed that the changes of occupation necessitated by the increasing disablement caused by the bronchiti explained the increased number of bronchitic patients in the lower social classes. One possible weak point in this investigation—if attempt *is to be made to* draw epidemi logical conclusio s—is that h pital admission as such *for thi particular* disease may have some relatio ship to the *ocial situ tion.*

Bentsen (1962) studied the sequelae of the *chronic ob tructive* pulmonary diseases i a Norwegian rural population. A person with ch onic obstru tive p lmonary disease was classified as disabled when th dyspnoea was persi tent nd so great that he was oppressed by walking at an rdinary pace The uthor pointed out that after the ge of 50 years the *disabling effect of chronic* obstructi e pulmonary disease increased with

ficant sex difference was found in this respect.

Ramgren (1962) made a study of the medico-social aspect of haemophilia in Sweden—in 233 persons altogether. The general education of these patients was investigated, and also the types of work by which they were able to earn their living. It was found that of these haemophilia patients only 60 had received an adequate education. One-third had been taught at special schools for the handicapped or had received instruction at home. Practically all of those whose work was of the intellectual or similar type were able to support themselves financially. A large number of those who had work of the light manual type however were unable to earn their living. 16 % or 38 patients in the entire series were receiving an invalid pension.

The marriage frequency and fertility in patients with mental diseases have been studied by several authors. Only three will be mentioned here, namely Essen-Möller (1935), Dahlberg (1942) and E. Johanson (1958), all of whom found a low marriage frequency and fertility rate. E. Johanson also studied the criminality in a series of male schizophrenic patients and found that there was no significant deviation from the expected figure in a normal population.

Socio medical aspects of other chronic diseases

Only a few large systematic investigations of the social aspects of other chronic diseases appear to have been made in Sweden Reference will be made here to a few investigations of such diseases in which at least some of the indicators of social situation (e g marriage frequency income level housing standard etc.) which were studied in the present investigation were used

Smårs and Berfenstam (1961) reported a systematic socio-medical tudy of 100 individuals with osteogenesis imperfecta. In this disease the incapacity obviously lies in impairment of movement The general school educati n had been sati factory In studying the different indicators of social situation, information from official statistics was often used f r comparisons. The authors found that the marriage frequency in their com plete eries of cases was l wer than for the total p pulation. For the group of patient who were les severely h ndicapped by th disea e it wa considered probable that the number of hil lren was only lightly below *that of the t tal population.* With reg rd to income the economically active did n t on an average differ to any noteworthy extent from the tot l population. At the time f th *in estigation 22 pati nt (18 /)* were re cei ing an in alid pension n account f osteogen I imperfecta The h u ing stan dard were good. The uthors summa l ed by pointing out the surp isingly good social dapt tion ften h wn by pati ts with a se ere handicap They con idered th t the funct onal impairment wa compensated to great ext nt by good mental balance *ad pt ti n to the r itu tion and* high intel lectual cap ity

Bruce Dahlström and Uggla (1960) made ocio-medical studies on patients from Stock l olm with an ther hronic illnes i. e. tube culosis. The mat rial comprised th 230 hos pital in patients suffering from thi disease

The marriage and divorce *frequencies and* housing conditions were among the indica tors of social situation studied in this in vestigation. The results were coloured, how ever by the large alcohol consumption among a remarkably large number of the patients. Because of this the material as a whole gave the impression of a poorer social situation as judged by the majority of the indicators studied.

Alcoholism also affected the social con dition found by Pegelow (1960) in his in vestigation on 339 consecutive patients re ceiving hospital treatment for active pulmo nary t berculosis. 27 / of the 240 men were classified as chronic alcoholics. This could have explained, for example the unsatis factory housing conditions found in a large number of cases, and also th fact that *public sistance relief wa being received by* 65 (26 %) of the males at the time of admis sion to hospital.

Sonja Rislager Calais (1960) studied the rehabilitation of polio-myelitic patients four years after the ons t of the disease in 1953.

Studies were made f the length f the period in h spital, the return to work a d occupation t the time of the investigation hanges of occupati by reason f the disease the re-training frequency the n m ber of unemployed economic changes, hou ing conditions and the frequency of invalid p nsioners.

In pite of d ice to hange th ir work and undergo re-trai ing o ccount of th ir paralysis, se eral had returned t their old occupations. Th soci l condition ppeared to be better amo g those who, i spite of their *handicap had f u d it possible t re* turn to thei f m r work probably due t the fact that re training often in olves a hange o er to m re poorly paid wo k.

Changes of occupatio accou t f poliomyelitis appea ed t be m re frequ t in the severely disabled categ ies. On assessing the relati ship between general ed cation nd ret rn to work it wa found that those who w re *f rced t change th* occupation had on *an a erag l wer ed* tion. At th tim f the i estigati n 17 were rec i i g n invalid pension (gal

should have been at least 16 years of age on admission to hospital.

The number of patients who had been admitted to hospital for bronchial asthma and fulfilled the above criteria was 176 (71 men and 105 women). These patients were sought in the Uppsala County population register, where it was found that 15 of them (8 men and 7 women) had died before the 1st November 1960. A further 21 (8 men and 13 women) had moved to another district before this date. The remaining 140 patients were contacted by post or telephone. One man was in hospital for mental diseases, for long term treatment, and could not therefore take part in the investigation. 4 patients (2 men and 2 women) refused to participate for other reasons. 3 patients (2 men and 1 woman) died and 4 (2 of either sex) moved to another district after the 1st November 1960 before the investigation was begun.

128 patients (48 men and 80 women) thus remained. These patients then underwent examinations, which mainly took place between September 1961 and April 1963. During this period all of the patients were examined in the out-patients department, and all with the exception of 7 women were admitted to wards for further investigation. Of the 128 patients 12 were diagnosed as having chronic diseases—apart from their asthma—which were considered to have had a decisive influence on their general condition. These 12 underwent the same investigations as the others, but were excluded from the general analyses of clinical and physiological data and those of socio-medical data with the exception of calculations made with regard to mortality and change of domicile. These latter calculations thus comprised all of the patients who on admission to hospital fulfilled the original criteria for inclusion in the material.

With the aid of the population register and the registry office contact was *obtained* either by letter or telephone with the majority of those who had moved to other places within Sweden in order to obtain information about their asthma. For those who died, information on the date and cause of death was obtained.

To summarize there were 176 patients who on admission to hospital during the given period fulfilled the original criteria for inclusion in the material. This figure corresponded to approximately 0.4 % of those persons in the population of the areas in question, who were born during the period 1893–1932. The number of those who fulfilled the final criteria for inclusion in the material and who were available for investigation was 128. Of these 12 were diagnosed as having or having had chronic diseases —apart from the asthma—which were considered to have had a decisive influence on their general condition. *There thus remained 116 patients who constituted the material on which the main part of the investigation was based (hereafter called the main material).*

Evaluation of the composition and completeness of the material

The aim of this investigation was to study and draw conclusions from a group of patients who had fulfilled certain given criteria, such as *age* limits and domicile, and who during a given period had been admitted to certain hospitals by reason of bronchial asthma. The material was thereby probably limited to comprise *mainly* severe and moderately severe cases of the disease. The completeness of the material will be treated below but firstly a *few questions regarding* the delimitation of the material will be discussed.

IV MATERIAL

In order to study the functional sequelae in patients with bronchial asthma it was considered appropriate to select the material from patients who during an exacerbation of the disease had attended a hospital where there were adequate possibilities of differential diagnosis.

Principles used in the collection of the material

The following principles were applied in the collection of the material

1 The patient who should have been resident in Uppsala or in one of seven surrounding communes should have been admitted to one of the Uppsala hospitals mentioned below during the years 1945–58 and should have been judged to be in need of hospital treatment because of bronchial asthma (176 patients hereafter called the total asthma series) This figure corresponds to approximately 0 4 % of the population of the investigation area in the same age groups The patient should still be resident within the investigation area in November 1960 (140 patients remain)

The patient at the time of dmi ion to hospital, should ha e been on the population register in Uppsala or one f the rural communes surrounding it (North and South Hagunda Bälinge, Björklinge, Rasbo Vaksala and Vattholma) By the limitation of the areas from which the patients were taken (hereafter called the collection area) it was considered probable that practically all persons in these areas who were in need of hospital treatment for bronchial asthma wo ld have been referred to hospitals situated in Uppsala. These hospitals would then be either the University Hospital (medical, lung, or ear nose and throat clinics) or the medical departm nt of the Samaritan Hospital.

2 The reason for admission to hospital should either be a current attack of bronchial asthma—there should be no grounds for suspecting any degree of cardiac asthma—or to carry out specific hyposensitization if at the acute stage the patient was being treated in the out patients department

Thus n pati nts were included for whom the actual reas f r admission was the treatment of foci of infection which were s pected of cau ing th asthma. The latter delimitation was made mainly because these patients often had a mild type of sthma that in itself did not necessil t hospital treatm t—in several f these pati nts the fore the diagnosi f a thma could not be accepted with certainty

3 The patient should have been born during the period 1893–1932 and

have varied during a period as long as 14 years, due to changes of doctors responsible for the admissions, and to variations in the number of beds available.

In no fewer tha 166 of the 176 quali-fied patients the Unic t which t least on one occasion they had f lfilled the re quirement f r admission—and thereby in lusion i the material—was one and the same i the Medical Clinic of th Uni-ersity Hospital, Uppsala. F rthermore this Unic had the same Head for 13 of the 14 years, nd it may therefore be assumed that the principles for dmission to thi department were retail ly nchanged during this period

During th period in question the frequency of admission for bronchial asthma in this clinic was practically tationary the increase in the popula tion of the areas served being taken int account It is therefore probable that any variations with respect to the indication for admi sion would ha concerned only patients with mild symptoms If the investigation permit a selection it would be the milder cases that would probably be excluded from the m terial

The mea nnual number of patient who during the first five years of the qualifica tion period f lfilled the criteria, an admi sion t hospital, for inclusion in the ma terial, was 12.4 The corresponding figu es for the second fiv year period nd for the emaining fou ears were 12.8 nd 11.3 especti l These figures pply to first t me adm ions during the period in ques-ion If re admissions are included the last t figures in particula ha to be cor rected to somewhat higher level. The population of persons born between 1890 nd 1932, the ollection rea, increased by pproximatel 2 during the qualifica-tion period"

This therefore supports the view that the indications for admission to hospital were relatively unchanged throughout this period.

The possibility of medical care being received outside Uppsala

The possibility that some patients might have sought medical care in hospitals outside Uppsala must exist. It may be regarded as improbable, however that an asthma patient faced with an acute situation, when hospital treatment is obviously indicated, would leave his own area and seek advice at a hospital some distance away It is conceivable, on the other hand, that this patient might attend such a hospital during free or rela tively free intervals.

Nine patients with asthma of a degree of severity such that the acute attack could be treated in an out patients department, and not neces sitate admission to a ward were in cluded in the total asthma series be cause they were later admitted to a ward for hyposensitization. There is a risk, but very small, that similar cases were missed from the recordings by reason of the fact that this hypo-sensitization was carried out in hospi tals situated outside the area served by the Uppsala University Hospital or pos ibly in an outpatients depart ment It must be regarded as highly improbable however with the present systems of medical service and the difficulties in arranging for medical care in other hospitals with special departments, that more than a few cases would have been missed in this way

Delimitation of the "collection period" to 1945–1956

It was chosen to collect the material from a limited period of time. Those patients being "qualified" for inclusion who during a certain period fulfilled certain prerequisites for admission to hospital. The possibility of a patient in the collection being "qualified" was obviously dependent upon the length of the "qualification period". By extending the period to as long as 10 years the size of the material was considered to be adequate. The intermittent nature of the disease with sometimes lengthy or partly symptom-free periods which in some cases may extend over several years motivated a relatively long qualification period so as to increase the chance of a patient being included in the material.

Delimitation of the years of birth to 1885–1925

By limiting the required years of birth to the given period it was supposed that some over-representation of older patients would be avoided. It seemed probable and at the same time it was presupposed that these patients had had an early onset—and that the disease in these older persons would have had greater possibilities of influencing the functional sequelae. As will be shown later the majority of patients were under 40 years of age at the onset of the disease, gradually fewer at a more advanced age, and the number with an early onset who still qualified for inclusion in the material was therefore limited.

It may be assumed, for example, that some of those who were born between 1900 and 1911 and who were admitted to hospital during the 1930s and first half of the 1940s would not have been so disposed later and would therefore not be included in the material. The correctness of this assumption is supported by the fact that some of the younger patients with an early onset who received hospital treatment during the latter half of the 1940s had not a permanent improvement. In the end of the 1950s that admission to hospital was necessary.

Lindeberg (1954) also found a relatively small number with an early age at onset in an adult material of patients with bronchial asthma. In her material there was a relationship between age and age at onset, a higher age being combined on an average with a higher age at onset.

Since one of the aims was to study the effect of the asthma on the social adaptation of the patients, it was considered desirable to set the lower borderline for the patients' age at a sufficiently high level for the patients to have worked for some years as an independent member of the community outside the parental home. In choosing the upper age limit consideration was taken of the fact that persons born in 1885 would have reached the age for old age pension in 1950. It was considered that for patients over this age some difficulties would have been encountered with regard to certain investigations and obtaining satisfactory comparison material.

Deviations in the indications for admission to hospital

It is obviously possible that the indications for admission to hospital could

have varied during a period as long as 14 years, due to changes of doctors responsible for the admissions, and to variations in the number of beds available.

[illegible] no fewer than 166 [illegible] f the 176 qualified patients the clini[illegible] t which at least on one occasion they had fulfilled the requirement for [illegible]dmission—and thereby inclusion in the material—w[illegible] one and the same [illegible] the Medical Clinic of the University Hospital, Uppsala. Furthermore this clinic had the same Head for 13 of the 14 years, [illegible]nd it may therefore be assumed that the principles for admission to thi[illegible] department were relatively unchanged during this period.

During the period in question the frequency of admissions for bronchial asthma in this clinic was practically [illegible]tationary, the increase in the population of the areas served being taken into account. It is therefore probable that any variations with respect to the indications for admis[illegible]ion would have concerned only patients with mild symptoms. If the investigation permit[illegible] a selection it would be the milder cases that would probably be excluded from the material.

The mean [illegible]nnual number of patient[illegible] who during the first fi[illegible] years of the qualification period fulfilled the criteria, on admission to hospital, for inclusion in the material, was 13.4. The corresponding figures for the second fi[illegible] year period [illegible]nd for the remaining four years were 12.8 [illegible]nd 11.3 respectively. These figures [illegible]pply t[illegible] first[illegible] ime admis[illegible]ion[illegible] during the period in question. If re[illegible]dmissions [illegible]re included the last tw[illegible] figures in particula[illegible] ha[illegible] to be cor[illegible]ected to [illegible] somewhat higher level. The population of person[illegible] born between 18[illegible] and 19[illegible], in the collection areas, increased b[illegible] [illegible]pproximately 2[illegible] during the qualification period.

This therefore supports the view that the indications for admission to hospital were relatively unchanged throughout this period.

The possibility of medical care being received outside Uppsala

The possibility that some patients might have sought medical care in hospitals outside Uppsala must exist. It may be regarded as improbable however that an asthma patient faced with an acute situation, when hospital treatment is obviously indicated, would leave his own area and seek advice at a hospital some distance away. It is conceivable, on the other hand, that this patient might attend such a hospital during free or relatively free intervals.

Nine patients with asthma of a degree of severity such that the acute attack could be treated in an out[illegible]patients department, and not necessitate admission to a ward, were in[illegible]cluded in the total asthma series because they were later admitted to a ward for hyposensitization. There is a risk, but very small, that similar cases were missed from the recordings by reason of the fact that this hyposensitization was carried out in hospi[illegible]tals situated outside the area served by the Uppsala University Hospital, or possibly in an outpatients depart[illegible]ment. It must be regarded as highly improbable however with the present systems of medical service and the difficulties in arranging for medical care in other hospitals with special departments, that more than a few cases would have been missed in this way.

Delimitation of the "collection period to 1945–1958

It was chosen to collect the material from a limited period of time those patients being "qualified for inclusion who during a certain period fulfilled certain prerequisites for admission to hospital The possibility of a patient in the collection area being qualified was obviously dependent upon the length of the qualification period By extending this period to as long as 14 years the size of the material was considered to be acceptable The intermittent nature of the disease with sometimes completely or partly symptom free periods which in some cases may extend over years motivated a relatively long qualification period so as to increase the chance of a patient being included in the material

Delimitation of the year of birth to 1893–1932

By limiting the required year of birth to the given period, it was supposed that some over representation of older patients would be produced It seemed probable—if at the same time it was presupposed that the asthma had had an early onset—and that the disease in these older persons would have had greater possibilities of influencing the functional sequelae. As will be shown later the majority of patients regardless of their age at the onset of the disease gradually show improvement and the number with an early onset who still qualified for inclusion in the material was therefore limited

It may be assumed, for example that many of those who were born between 1900 and 1915 and who were admitted to hospital during the 1930's and first half of the 1940's, would not have returned later and would therefore not be included in the material. The correctness of this assumption is supported by the fact that many of the younger patients with an early onset, who received hospital treatment during the latter half of the 1940's, had such insignificant symptoms by the end of the 1950's that admission to hospital was unnecessary

Fagerberg (1958) also found a relatively small number with an early age at onset in an adult material of patients with bronchial asthma In his material there was a relationship between age and age at onset a higher age being combined on an average with a higher age at onset.

Since one of the aims was to study the effect of the asthma on the social adaptation of the patient it was considered desirable to set the lower borderline for the patient's age at a sufficiently high level for the patient to have lived for some years as an independent member of the community outside the parental home. In choosing the upper age limit, consideration was taken of the fact that persons born in 1893 would have reached the age for old age pension in 1960 It was considered that for patients exceeding this age some difficulties would have been encountered with regard to certain investigations, in obtaining satisfactorily comparative material

Variations in the indications for admission to hospital

It is obviously possible that the indications for admission to hospital could

have varied during a period as long as 14 years, due to changes of doctors responsible for the admissions, and to variations in the number of beds available.

I no fewer than 168 of the 176 qualified" patients the clinic t which t least on one occasion they had f lfilled the quirements for admission—and thereby inclusion in the material—was one and the same i. the Medical Clinic of th University Hospital, Uppsala. Furthermore this clinic had the same Head for 13 of the 14 ears, nd it may therefore be assumed that the principles for admission to thi department were relatively nchanged during this period

During the period in question the frequency of admissions for bronchial asthma in this clinic was practically stationary the increase in the popula tion of the areas served being taken into account. It is therefore probable that any variations with respect to the indications for admission would ha e concerned only patients with mild symptoms. If the investigation permit a selection it would be the milder cases that would probably be cluded from the material.

The mean nnual number of patients who during the first fiv years of the qualifica tion period fulfilled the criteria on dmission to hospital, for inclusion in the material, w 13.4 The corresponding figures for the second five- ear period nd for the remaining four ears were 12.8 nd 11.3 respectiv l These figures pply to first me adm ons during the period in question. If re admissions are included the last tw figures particula hav to be cor ected to somewhat higher level. The population of persons born between 1893 nd 1932, the collection reas, increased by pprox mately 3 during the qu l fica tion period"

This therefore supports the view that the indications for admission to hospital were relatively unchanged throughout this period.

The possibility of medical care being received outside Uppsala

The possibility that some patients might have sought medical care in hospitals outside Uppsala must exist. It may be regarded as improbable, however that an asthma patient faced with an acute situation, when hospital treatment is obviously indicated, would leave his own area and seek advice at a hospital some distance away It is conceivable, on the other hand, that this patient might attend such a hospital during free or rela tively free intervals.

Nine patients with asthma of a degree of severity such that the acute attack could be treated in an out patients department, and not neces sitate admission to a ward, were in cluded in the total asthma series be cause they were later admitted to a ward for hyposensitization. There is a risk, but very small, that similar cases were missed from the recordings by reason of the fact that this hyposensitization was carried out in hospi tals situated outside the area served by the Uppsala University Hospit l, or possibly in an outpatients depart ment. It must be regarded as highly improbable however with the present systems of medical service and the difficulties in arranging for medical care in other hospitals with special departments, that more than a few cases would have been missed in this way

Delimitation of the collection period to 1945–1958

It was chosen to collect the material from a limited period of time those patients being "qualified for inclusion who during a certain period fulfilled certain prerequisites for admission to hospital The possibility of a patient in the collection area being qualified was obviously dependent upon the length of the qualification period By extending this period to as long as 14 years the size of the material was considered to be acceptable. The intermittent nature of the disease with sometimes completely or partly symptom free periods which in some cases may extend over years motivated a relatively long qualification period" so as to increase the chance of a patient being included in the material

Delimitation of the year of birth to 1893–1932

By limiting the required year of birth to the given period it was supposed that some over representation of older patients would be produced It seemed probable—if at the same time it was presupposed that the asthma had had an early onset—and that the disease in these older persons would have had greater possibilities of influencing the functional sequelae As will be shown later the majority of patients regardless of their age at the onset of the disease, gradually show improvement, and the number with an early onset who still qualified for inclusion in the material was therefore limited

It may be assumed, for example, that many of those who were born between 1900 and 1913 and who were admitted to hospital during the 1930 s and first half of the 1940 s, would not have returned lat r and would therefore not be included in the material. The correctness of this assumption is supported by the fact that many of the younger patients with an early onset, who received hospital treatment during the latt r half of the 1940 s, had such insignificant symptoms by the end of the 1950 s that admission to hospital was unnecessary

Fagerberg (1958) also found a relatively small number with an early age at onset in an adult material of patients with bronchial asthma In his material there was a relationship between age and age at onset a higher age being combined on an average with a higher age at onset

Since one of the aims was to study the effect of the asthma on the social adaptation of the patient it was considered desirable to set the lower borderline for the patient s age at a sufficiently high level for the patient to have lived for some years as an independent member of the community outside the parental home. In choosing the upper age limit, consideration was taken of the fact that persons born in 1893 would have reached the age for old age pension in 1960 It was considered that for patients exceeding this age some difficulties would have been encountered, with regard to certain investigations, in obtaining satisfactorily comparative material.

Variations in the indications for admission to hospital

It is obviously possible that the indications for admission to hospital could

have varied during a period as long as 14 years, due to changes of doctors responsible for the admissions, and to variations in the number of beds available.

In no fewer tha 160 of the 176 qualified patients the clinic at which at lea t on one occasion they had f lfilled the re quirements for admission—and thereby inclu sen in the material—was on and the sam L the Medical Clinic of the University Hospital, Uppsala. Furthermore thi clinic had the same Head for 13 of the 14 years, nd it may therefore be assumed th t the principles for admission to thi department were relati ely nchanged during thi period.

During the period in question the frequency of admissions for bronchial asthma in this clinic was practically stationary the increase in the popula tion of the area served being taken into account It is therefore probable that any variations with respect to th indication for admission would have concerned only patients with mild symptoms. If the investigation permit a selection it would be the milder cases that would probably be cluded from the material.

The mea annual number of patient who during the first five years of the qualifica tion period f lfilled the criteria, on admission to hospital, for inclusion in the material, wa 13.4 The corresponding figures for the second fi ear period and for the remaining four ears were 12.6 and 11.3 respectiv (These figures pply t first time admissions during the period in question If re dmissions are included the last tw figures in particular ha to be cor rected t somewhat higher lev l. The population of persons born between [illegible] nd [illegible], in the collection areas, increased by pproximately 3 during the qualifica tion period

This therefore supports the view that the indications for admission to hospital were relatively unchanged throughout this period.

The possibility of medical care being received outside Uppsala

The possibility that some patients might have sought medical care in hospitals outside Uppsala must exist. It may be regarded as improbable, however that an asthma patient faced with an acute situation, when hospital treatment is obviously indicated, would leave his own area and seek advice at a hospital some distance away It is conceivable on the other hand that this patient might attend such a hospital during free or rela tively free intervals.

Nine patients with asthma of a degree of severity such that the acute attack could be treated in an out patients department, and not necessitate admission to a ward, were in cluded in the total asthma series be cause they were later admitted to a ward for hyposensitization. There is a risk, but very small, that similar cases were missed from the recordings by reason of the fact that this hyposensitization was carried out in hospi tals situated outside the area served by the Uppsala University Hospital, or possibly in an outpatients depart ment. It must be regarded as highly improbable however with the present systems of medical service and the difficulties in arranging for medical care in other hospitals with special departments, that more than a few cases would have been mi sed in this way

The possibility of medical care being received at home

The possibility of some patients receiving medical care at home even during severe acute attacks is another factor that should be considered. As in the cases just referred to, the likelihood of whether or not hospital care is sought may bear a certain relationship to the degree of severity of the asthma, and it may therefore be regarded as probable that the larger proportion of the patients with pronounced symptoms became "qualified". There is reason to assume that home nursing under the supervision of a doctor would only occur in special social groups, so that if there had been some patients receiving medical care in this way, there would have been a certain degree of selection in the material. It is my opinion, however, that no more than a few isolated cases could have been omitted in this way.

The correctness of the diagnosis of bronchial asthma

Since the primary criterion for a patient's inclusion in the material was that he had attended hospital for acute bronchial asthma and that this acute situation was either a direct reason for his admission to a ward (this applied to 167 patients) or an indirect (this applied to 9 patients), there appears to be no reason for questioning the correctness of the diagnosis, since in most cases there is no difficulty in diagnosing this disease on observation and examination during an actual attack.

One of the main difficulties in diagnosing the symptoms at the acute stage is their differentiation from the symptom picture shown by patients with left cardiac incompensation. Other causes of dyspnoea, e.g. intra- or extra-pulmonary neoplasm, may give rise to a type of respiratory obstruction that may possibly lead to problems in differential diagnosis. In these cases, however, it is highly probable that the patients would return to hospital at a later date and the conditions would be diagnosed then. For details in the differential diagnosis reference may be made to Unger (1952).

The following facts support the correctness of the diagnosis of bronchial asthma in this series of patients.

a) the onset of acute symptoms was as a rule followed directly—or in exceptional cases after a short period—by admission to hospital, where in any doubtful cases differential diagnosis was possible.

b) careful study of the case histories left the author in no doubt that all the patients in this material had undergone periods of symptoms typical of bronchial asthma.

Discussion on the evaluation of other complicating chronic diseases and the classification of patients with such diseases

Many of the patients had at some time suffered from a disease other than asthma; this was either transitory or of a chronic type. An attempt was made from case to case to estimate any influence which this intervening disease might have had on the respiratory and circulatory functions and on the social adaptation of the patient. It was also considered important from a socio-medical aspect

to take into account the fact of whether the disease coincided with a period especially sensitive with regard to social adaptation. It was obviously impossible to establish absolute norms, partly because of the subsequent difficulties in analysing the influence of these diseases, and partly because these supervening diseases were so varied.

The patients concerned here were divided into two groups. One group comprised those in whom the supervening disease was considered to have had a decisive influence—this group was therefore excluded from the general analyses. The other group which was included in the main material comprised patients in whom no uch influence appeared probable. In all except two or possibly three patients in the first group however it was the bronchial asthma that had had the greatest influence on the length of the period of disablement and on the lif situation in general. Nevertheless all patients belonging to this group were ex luded from the analyses concerning clinical and physiological data, and also those concerning socio-medical data with the exception of the calculation on mortality and change f domicile. It was considered justified to include the patients with other chronic diseases in these two latter nalyses, ince neither those who had moved nor those who had died, in the a thma series, were investigated in detail for the possible occurrence of other diseases. The classification of the patients into the two groups according to the clinical assessment is given in an appendix (page 160)

Patients excluded from the material because they had moved from the collection area

The 26 persons who had moved to another district did not take part in the main investigation, but were on the other hand included in the calculations of mortality nd change of residence. These patients were ex luded from the socio-medical analyses because otherwise it would ha been necessary to obtain special comparative material for practically each one of them, from the place or places in which they had li ed afte moving from the collection area. It was not considered justifiable to include them in the investigations on respiratory and circu latory function, partly because of the cost involved in carrying out examinations t hospitals outside patient' own county Since however will be shown later the frequency of change f resid nce was com paratively low nd there wa no reason t assume that the mea severity of the thma of these persons differed notably from that of those who had not moved from the collec tion area, the results of the main investiga tions were probably not affected significantly by the exclusion of these patients. I an t tempt to confirm the probability that these patients, from a case history point of view did not constitute extrem group contact by post or t lephone, with the id f the population register and registry office, wa made with the majority of those who were till liv nd living in Sweden.

V METHODS

The description of the method will be divided into three sections. These sections treat

1 Methods for assessment of clinical course with respect to the degree of severity of the bronchial asthma.
2 Methods for analysis of circulatory and respiratory function.
3 Principles of classification in the treatment of socio medical problems

Assessment of clinical course with respect to the estimated degree of severity of the bronchial asthma

Personal interviews with the patients were carried out by the author mainly during the period August 1961—December 1962 and were based on a previously prepared questionnaire comprising questions on both personal data and the history of the patient's illness On taking the case history special importance was placed on the estimated number of days of incapacity and symptom days since the onset of this disease By day of incapacity is meant a day when owing to asthma there was inability to work estimated as at least 75 % and by symptom day at least 25 % It is obvious however that this method of assessing the number of days of incapacity and symptom days only allowed a rough estimate. Poor memory on the part of the patients for dates that were a relatively long time back, could have been considerable. There appeared however to be no better mode of procedure.

If the recordings that are now to be found in the General Sickness Insurance had extended further back than 1955 it would have been possible to obtain certain information regarding the days when sickness benefit was received. Such information, however would have been of limited value owing to the intermittent course of the disease because of the number of days that must lapse before application is made for sickness benefit this application is often not made when the symptom periods are short In spite of this some information could however have been gained from the old sickness benefit service and other benefit funds, but as mentioned above this material was not available for the earlier part of the period.

Attempt was made to compare the information received from the patients with that obtained from the General Sickness Insurance which applied to the period 1955–1960 It was found, however that there was a discrepancy between the days of incapacity noted in the Insurance records and those found in this investigation As previously mentioned short term illnesses were not always found in the records of the General Sickness Insurance.

Furthermore discrepancies obviously occurred between the recorded number of days when sickness benefit was received and the number of days for which the patient stated that he was disabled to an extent of at least 75 % on account of asthma. One reason for the difference may be that the period for which sickness benefit is obtained often includes convalescence days.

By dividing the tim after the onset of asthma into periods of a maximum of 5 years, it seemed possible at the interviews to form a good idea in all cases of the effect of the disease with regard to days of incapacity and symptom days. It was particularly difficult however to estimate the number of incapacity and symptom days for those persons who had the type of work in which absence owing to ickness does not result in loss of incom e.g. pensioners and housewi es. In these cases n assessment was made of the number of incapacity and symptom days that the person in question would have had if he or she had had light, mainly sedentary work.

Assessment of the degree of severity of the disease wa made by ascribing t ea h patient a number of sickness point which meant that each day of incapacity was equivalent to 1 point, and each ymptom day / point. The term over all degree of severity will be ubsequently used to denote the total number of sickness points since the onset of the disease. The mutual ev luation between day of incapacity nd symptom day was obviously somewhat arbitrary but this assessment of their gradation was considered reasonable.

The total number of asthmatic ttacks wa estimated analogously by the study of each period in turn. The erage du tion f the ttack wa determined by means of information from the patient to which of the f llowing groups the majority of the ttacks could be signed

Group 1 ttacks lasting for les than 1 hour

Group 2 attacks lasting 1 3 hours.

Group 3 attack lasting 3–12 hours.

Group 4 attacks lasting 13–23 hours.

A rough estimat of the total number of ttack hours wa obtained by multiplying the stimated total number of ttacks by the mea figure corresponding t the duratio group t which the patient was signed. Thus patient who was estimated having had 500 ttacks, the duration of most of which was 3–12 hours, had 500 × 7.5 = 3,750 attack hours.

The usual general medical examination was performed by the author personally—as in the case of the interview—both in the out patients department and during the patient's period in a ward in connection with the follow up examination. These examinations are described in more detail below where a summary of the interview and general examination scheme is given.

S mmary f questions included in the questionnaire

1. *The patient' name, date f birth, present address, h* marital *state and occupation*
2. The yea f onset of the bronchial sthma
3. The general course of the sthma (improvement deterioration or no change) since it onset.
4. Estimation f the total number of day of incapacity and symptom day on account of the thma.
5. Estimation of the total number of th matic attack nd thei erage duration.
6. The number of times dmitted t hospital on account of the thma.

7 The occurrence of chronic bronchitis in the case history

8. Information regarding any other diseases reported in the case history

9 Information regarding smoking and alcohol consumption.

Brief description of the general clinical examination Physical examination including an assessment of the general condition and body build measurement of height and weight recording of any signs of cardiac decompensation, measurement of blood pressure inspection of mouth and throat palpation of thyroid gland superficial lymph glands and abdomen, auscultation and percussion of heart and lungs and examination of arm and leg reflexes. Examination of blood status comprising Hb content, hematocrit sedimentation rat and differential count of white blood cells. Examination of urine comprising qualitative albumin and glucose t sts and sedimentation.

The results of some of these clinical examinations are not reported, since they served nly for sorting the material For examj le investigations intended to test ertain functions were postponed if pronounced anaemia was found in any of the patients. Treatment of the anaemia was commenced and th patient returned when this was cured Tests of physiological functions were performed if in the women the Hb content was not les than 10.5 g /s, and in the men not les than 11.5 g /s. Women with a Hb content of less than 11.5 g / and m n with a Hb cont nt of less than 12 5 g % are noted on page 161

The I f rm ati n gi n in points 7 and 8 of the questi nnaire facilitated sorting out those patients with a ther chronic disease —in addition to the bronchial asthma—whicl was judged to ha e had a decisive i fluence on tl patient s general situation tate of health o functions.

In order to demonstrate changes in the estimated degree of severity of the disease, the average number of sick ness points per year was studied for each patient during the period 1957–1961 and this figure compared with the corresponding figures for the first five years after the onset of the dis ease, and the *period 1947–1951* re spectively and also with the average *figure for the whole period* between the onset of the disease and the end of 1956 The course of the disease in those patients who during early and late periods respectively attained either relatively low or high sickness points was studied. This is discussed in more detail in the reports of the results on page 35

Analysis of circulatory and respiratory function

In order to avoid functional changes resulting from a recent asthmatic attack the tests of respiratory and circulatory function were made during a free interval The latter means that during the period of the tests and for at least two days previously the patient was either entirely free from *symptoms or in what he regarded as* his most symptom free state for the last *twelve months On auscultation of the* lungs during normal breathing there should either be no rhonchi heard or the above-mentioned criterion for the most optimal state should apply

No symptom tic therapy (with the e ception of corticosteroids in those patient who had had l ng term therapy L daily doses f r the last six months) should ha e been taken during the 24 hours before testing began. After th work and l ng fu cti tests were completed the patients received two inhalations of Isoprenalin pray (Medihaler—Hässl)

Electrocardiogram. ECG was recorded at rest in the supine position, after eight minutes of passive standing, during exercise in the sitting position, and after exercise in the supine position. The following leads were used: I, II, III, aVR, aVL, aVF, V_1, V_2, V_4, V_5 and V_6. During exercise the limb electrodes were moved to the forehead. The ECG-apparatus used was Mingograf 42 (Elema Schönander Ltd, Stockholm).

BMR. Basal oxygen uptake was determined under standardized conditions during ten-minute periods, with duplicate determinations on each of two separate occasions. The apparatus used were Spirograf Krogh and "Spirograf IV" (Elema Schönander Ltd, Stockholm); this equipment was described by Å estrand and Bj lkland (1961).

Physical work capacity. The work test (Sjöstrand, 1947; and Wahlund, 1948) was performed on an electrically braked bicycle ergometer (Holmgren and Mattsson, 1954). The heart rate and respiratory frequency were counted, and the ECG was recorded continuously during stepwise increases of work load. For the women the test was started at a rate of 200 kpm/min and for the men at 300 kpm/min. Each work period lasted for six minutes and the work was increased in steps of 200 and 300 kpm/min, respectively, until a heart rate of approximately 170 beats/min was reached or until the appearance of some abnormal signs or symptoms the work test was discontinued. The physical work capacity is arbitrarily defined as the absolute work load performed during a steady state at each of the heart rates 170, 150 and 130 (W_{170}, W_{150} and W_{130}). Steady state is defined either as 10 beats/min or less change of heart rate from the second to the sixth minute of work, or as 2 beats/min or less change from the fourth to the sixth minute. If steady state was not reached, the values for W_{170} etc. were calculated nevertheless, but are specially indicated in the tables and figures. The value of work capacity was obtained by numerical extra- or interpolation using the approximately linear relationship between heart rate and work load; extrapolation was not performed for more than 20 beats/min. The highest work load actually performed by the patient for at least 6 min is designated W_{max}. The highest heart rate actually attained during the highest work load is designated HR_{max}.

The majority of the patients were tested on two separate occasions, mostly within the course of six months. Both for W_{130} and W_{max} the mean value is given for the first and second occasion, separately. When no regard was taken of sex the mean value of W_{130} was 402 kpm/min at the first test and 426 kpm/min at the second test. The corresponding values for W_{max} were 858 kpm/min and 845 kpm/min, respectively. The standard deviations of the differences between the first and second tests, were for W_{130} 95 kpm/min and for W_{max} 87 kpm/min. The error of the single determination was therefore 67 kpm/min for W_{130} and 62 kpm/min for W_{max} (corrected for the difference in means). For W_{130} the mean difference between the first and second work test was 24 kpm/min, which means that there was a probable systematic error (?). For W_{max} the systematic error was not of a significant order of size. It is noteworthy however that the errors did not lie in the same direction.

The predicted average normal value for rate of work at heart rate 130 (W_{130}) and maximum performed work (W_{max}) was calculated for the patients in the asthma series from the values and equations given below. These were obtained from an analysis of normal data from the City of Uppsala Health Survey (1961) (Irnell and Linder, unpublished observations: age 51–66 years, 35 men and 61 women).

Men

average W_{130} = 650 kpm/min (SD = 158 kpm/min; SE = 22 kpm/min)

average W_{max} = 1722–14 x, where x is the age of the patient in years

(SD = 246 kpm/min; SE = 33 kpm/min)

Women

average W_{130} = 411 kpm/min (SD = 118 kpm/min; SE = 15 kpm/min)

average W_{max} = 1363–14 x, where x is the age of the patient in years

(SD = 157 kpm/min; SE = 20 kpm/min)

Heart volume. The heart volume was determined in the prone position, with the tube

above and the film below the patient For *the frontal projection the central ray was* angled This was originally suggested by Kjellberg Rudhe and Sjöstrand (1949) in their modification of the method of Larsson and Kjellberg (1948) These authors displaced the tube cranially to an angle of 30 to the vertical line In the present investigation a displacement in the caudal direction of 30 to the vertical line was tried. The result of this modification was empirically compared to that of the method of Kjellberg et al. (1949) In 36 of the present cases, chosen at random from the whole main material, the average *value for the tot l heart volume in the* prone position measured according to the present modification, was then found to be 6.0 ± 1.8 % greater than the average value obtained by the method of Kjellberg et al. (1949)

Right heart catheterization. The heart catheterization wa usually the last in estiga tion Th patients had been trained previously to such procedures as breathing through a mouth piece into a Douglas bag and cycling on a bicycl rgometer One hour before the investigation started the patient recei ed a small oral dose of pentymal (0.10 g if his w ight was below 70 kg and 0.15 g if it was bove) Right heart catheterization was per formed with th usual technique usually *from a left medial cubital vein. The patient* lay in the supine position during the whole procedure which included a graded work test on the bi ycle ergom ter A doub le-lumen catheter wa used A polyethylene cathet r wa placed in the brachial art ry Aft r thes proced res were completed the pati nt rested for approximat ly 15 minutes Pres ure were then recorded in the bra hial artery th right ent icle the pulm nary art ry and the *pulmonary wedge positi n (PCV pressur)* Ca diac output was determi ed according to the direct Fick principle E pirat ry air wa collecte l tarting three mi tes after the ins rti n of th m th piece and lasting f r t n minutes P es ure recordings were made d blood samples taken during the fifth to eighth minutes of gas collecti n. When these p ocedure were complet the patient rested again for approximately 15 minutes.

Each alternate patient then received a infusion of acetylcholine into the pulmonary artery with continuous recording of blood pressures. The dose rate was increased stepwise until a value of about 11 mg/min was reached *Constant infusion of this dose was* made for 15 minutes. Expiratory air was collected during the 5th to 15th minute of infusion. The pressures were measured and blood samples taken during the 8th to 12th minute

Following this procedure the patient rested for approximately 20 minutes before breathing a mixture of about 11 per cent oxygen (mean 11.32 %, lowest 11.20 %, highest 11.40) in nitrogen for 18 minutes. Expiratory air was collected during the 8th to 18th minute Pressures were recorded and blood samples were *taken during the 13th to 17th minute*

After this procedure the patient rested again for approximately 20 minutes and then *breathed a mixture of about 50 per cent oxygen* (mean 49.27 %, lowest 47.25 %, highest 52.28 %) in nitrogen. The procedure was the same as during anoxic anoxia.

After a further resting period of approximately 20 minutes each lternat patient, i.e those who were not gi en the i fusion of acetylcholine breathed a mixture of about 5 per cent carbon dioxide (mea 5.30 %, lowest 5.11 %, highest 5.88 %) in ir After an equilibration period of *5 mi utes, pressures were* recorded, blood samples taken and piratory air ollected d ring a period f a a rule 5 mi utes (rang 4—8) The pati t had a new resting period f pp mately 20 minutes and then the whole proced re was fi ished with a graded work test as a rule for women 200 and 400 kpm/min nd f r men 300 and 600 kpm/min. Each work load lasted for 6 min tes unless beca se of abnormal *sign o symptoms* the work test had to be ended The heart rat was determinded and press res recorded very second mi at Blood samples nd piratory air wer collected d ring th 4th to 6th min t of wo k load

Lung functi n t ts. The pirometer used (model Spirok mi l kif Ltd, Stockh lm) was a slightly modified versi n (Berglund, Birath, Bjure Grimby Kjellmer Sandqvi t and Söderh lm 1963) of that described by Bernstein, D'Silva nd Mead l (1952) Th

procedures for lung volume determination and ventilation capacity were essentially a described by Bergland et al. (1963), Birath, Kjellmer and Sandqvist (1963) and Grimby and Söderholm (1963). Nearly all the patient were tested on two diff rent occasion with an interval of ap tele 4—6 months. I the determination of the ital capacity (VC) forced expiratory volume in one second ($FEV_{1.0}$) nd maximum voluntary entila tio with fixed respiratory frequency of 40 per minute (MVV_{40}) or with a respiratory frequency chosen by the patient (MVV_f), nearly every patient performed each test twice on the first occasion nd three times on the second occasion. By FEV% is meant $FEV_{1.0}$ expressed percentage of VC or FVC (forced vital capacity) whichever wa the largest $FEV_{1.0}$ w estimated t the point of the spirogram occurring 0.10 second after the first expiratory deflection, whether the curv immediately assumed its teepest slope or showed initial part of flow acceleration. Before the investigation bega the patient was carefully instructed and was llowed initial trial run

All volumes are given t body temperature nd ambient pressure saturated (BTPS). All pirometers were operated by trained nurse. The international nomenclature of lung volumes and test were used

Since the aim of the investigation was to determine the maximum function, the mea wa not calculated. Each case is represented by the largest lue btained at either the first or the second examination.

Determination of total lung capacity (TLC) nd its sub-divisions was performed with the helium dilution method using closed spirometer (model Spirokemi, KIF Ltd, Stockholm). This investigation, however, in contrast to the other spirometer tests, was performed only once on each patient. The error f measurement of functional residual capacity for this method has been given by Holmgren (1954) 80 ml and by Grimby and Söderholm (1963) as 7.5 %.

For the thma patients the expected normal lues for ventilation capacity and lung volume were calculated from the regression equations (see Table 1) obtained from nor mal Swedish material, published by Berglund et al. (1963), Birath et al. (1963) and Grimby and Söderholm (1963). Their values, however were given t ambient temperature nd pressure saturated (ATPS). As the widest given range of room temperature and barometric pressure in these experiments was 18°—24 and 740—780 mm Hg, respectively the volumes t BTPS were obtained by multiplying the olumes t ATPS by factor of 1.08—1.12. The factor 1.10 wa hosen.

	Sex	Regression coefficients Age, yrs.	Height, m	Weight, kg	Constant	RSD
VC (l)	M	−0.026	+ 4.81	—	− 2.81	0.50
	F	−0.022	+ 4.04	—	− 2.33	0.40
FEV (l)	M	−0.033	+ 2.44	—	− 1.60	0.46
	F	−0.027	+ 2.87	—	− 0.54	0.36
FEV	M	−0.373	—	—	+ 91.79	7.19
	F	−0.381	—	—	+ 92.11	5.44
MVV_{40} (l/min)	M	−1.369	—	—	+180.6	18.6
	F	−0.818	—	—	+113.1	15.1
MVV_f (l/min)	M	−1.42	+79.0	—	+ 78	20
	F	−0.77	—	—	+128	20
TLC (l)	M	—	+ 6.92	−0.017	− 4.30	0.67
	F	−0.018	+ 6.72	—	− 3.77	0.48
FRC/TLC (%)	M	+0.18	—	−0.12	+ 32.2	6.8
	F	+0.18	—	−0.08	+ 43.3	4.7
RV/TLC (%)	M	+0.20	—	−0.14	+ 23.4	4.3
	F	+0.26	+27	—	− 25.0	4.5

Table 1. *Regression equations and residual standard deviations (RSD) for each lung volume measurement the control material*

Source: Grimby and Söderholm (1963)

In the present investigation, as in those of the last mentioned authors, the careful mea surement of *recorded volumes* was *not* performed until after the end of a patient's examination The difference between dupli cate or triplicate checks was thus not deter mined exactly during the examination. An examination was therefore not pursued until two identical maximum values were reached The effect of this procedure was analysed by calculating the difference between the *highest and the second highest value for each* test—whether it was registered on the first or the *second occasion. This difference* amounted on an average to 6 /₀ of the maxi mum value for VC, 8 % for $FEV_{1.0}$, 8 % for FEV%, 9 % for MVV_{40} and MVV_{f} In the control material (Berglund et al 1963) the above calculated difference amounted t an average of 1—3 /₀, and was theref re lower

Analysis of blood samples

Hemoglobin concentrati n (Hb conc) mg/100 ml was measured spectrophotometri cally as cyanmethemoglobin using Acul te s lution (Ortho Pharmaceutical Corporation New Jersey) Triple determinations were regu larly made The error of a single determina tion, calculated from all triple determinations was 1 01 per cent (at an av rage Hb conc of 13 1 g per 100 ml blood) In 111 of 794 sam ples one of the *triple determinations diff red* considerably (i e by more than 0.3 g/100 ml) *from the other two* In such cases the deviant value was rejected the result being calculated from the other two values. When the deviant values were excluded the err r of the method wa 0.84 per cent.

Oxygen saturation (S_{O_2}) was d termined by pectrophotometry of hem lysed whole blood (double determinations) as described in detail by Holmgren and Pern w (1950) The erro f a *single determination* was 0.0025 units at n a erage f 1,280 units *(unit = rati* of the absorbances at 475 nm and 505 nm) The error orrespond to an a erage value of 0.28 per c nt saturation, if the average xygen saturation f the blood samples w s about 75 per cent.

Oxygen capacity was calculated from the Hb concentration, using a value of 1.34 ml O_2/g hemoglobin. *Oxygen content* was calculated from the determined values of oxygen saturation *and oxygen capacity* with *the* addition of physically dissolved oxygen.

The *lactate concentration in capillary blood* was determined (double determinati ns) spectrophotometrically (Barker and Summerson, 1941 as modified by Strõm, 1949) The rror of a single determination was 6 8 per cent at an average lact te concentration of 6.1 mE per litre blood.

The plasma volume was determined by the method of dye dilution as described in detail by Wiklander (1956) (single determinati ns) Evans blue dye (T 1824) was injected intraven*ously and blood samples for spectrophoto*metric determination were taken at pproxi mately 10 and 60 minutes aft r the injection. Corrections for loss of dye to the extravas cular space (from time zero) and for the expected diff rence between bod hematocrit and peripheral hematocrit (ratio 0 90) were carried out. The error of a single determina tion was 3 1 per cent, a calculated from the *individual differe ces between* the 10-minute an l 60 minute values, at an average plasma volume of 2.54 litres. The error of the spectrophot metric analysis of a single blood sample was 5 per cent

The *hematocrit* was determined n microcapillaries (Int rnational Hemacrit Centrifuge International Equipment Company Boston) at 10 000 r p.m for 5 minutes N correc tion *for trapped plasma was made* s with this method it is negligible (Garby nd V lle 1961)

Principles of classification in the treatment of socio-medical problems

Summary of th questi nnaire used in the interview concerning the ocio-medical adaptati n f the patient Each patient wa ked *for information concerning*

1 General education.
2 Family f rmation (marriage t i l um ber f children and number under 16

years of age on the 1st November 1960- divorce)

3. Any change of residence and whether this was because of asthma.

4. Any change of occupation and whether this was because of asthma.

5. Whether the patient had discontinued income-yielding work and whether the reason for this was asthma.

6. The patient's housing situation on the 1st November 1960, both as regards size and standard.

7. Whether invalid pension was being received and whether this was because of asthma.

8. Whether any public assistance money was being received, and whether this was on account of the asthma.

9. The patient's own opinion of his capacity to work during the last year with regard to the asthma.

10. Smoking and alcohol consumption.

Change of domicil. For those members of the population of Uppsala, who on admission to hospital fulfilled the criteria for inclusion in the material, the number who had moved to other district before the 1st November 1960, the number still living in the collection area at this date and the average number of observation years were determined. The number still living in the Uppsala area during the observation period in question was also determined. The Uppsala population was used as control material. By studying the population schedules it was possible to estimate the number who during a certain observation period were still resident in Uppsala. The proband in both the asthma series and the control material were classified according to sex and age. The method is described in more detail on page 119.

Mortality. Information on the number who had died in the total asthma series was obtained from the registry office and population register. In estimating the mortality risk the town population of Sweden was used as control material, for which information was obtained from the official statistics. The proband both in the asthma series and the control material were classified according to sex and age. The method is described in more detail on page 168.

General education, family formation, number of children below the age of 16 years, distribution according to branch of industry and occupational status, income-earning among married women, smoking and alcohol consumption. A short description of the methods is given together with the corresponding reports of the results (cf. also Appendix 1).

Housing standard and size and income level in 1955 and 1960 according to the distribution into sickness benefit classes by the General Sickness Insurance. It was desired to compare the asthma series with the rest of the population in the collection area (hereafter called the Uppsala population). It was known that there were differences in age, sex and civil state between the asthma series and the Uppsala population. Since these factors were correlated both to the sickness benefit class and to the housing size and standard, each individual was ranked in the respective age, sex and civil state groups in the following way.

As an example the procedure in "ranking" with regard to the housing size (= number of rooms) for a particular person may be described. We take a married man born in 1918, who had a flat consisting of 2 rooms and kitchen. To determine his rank, a table from the Uppsala Housing Census for the 1st November 1960 is utilized. This table shows that of the married men aged 40–44 years, living together with their wives 936 (44.9 %) live in flats consisting of 2 rooms and kitchen or less, 648 (31.1 %) in flats of 3 rooms and kitchen and 501 (24.0 %) in flats of 4 rooms and kitchen or more. Those living in 3 rooms and kitchen, of the exemplified person's age, sex and civil state group, should be placed, in consideration of the housing size between 44.9 and 76.0. For the sake of simplicity he may be assigned to the middle of this interval, i.e. $\frac{44.9 + 76.0}{2} = 60.5$. This means that 60.5 % of the Uppsala population are considered to have inferior and 39.5

more favourable housing conditions as regards size. By this procedure each person was characterized by a percentage figure. If this figure was lower than 50 it implied that the person had less favourable conditions than the mean person in the same age, sex and civil state group. Married female patients living together with their husbands, were assigned according to the age of their respective husbands.

Analog us calculations were made with regard to the di tribution into sickness benefit classes. As in the study of the housing standard and size the urban district of Uppsala was treated separately from the rural districts around Uppsala, included in the investigation. The calculations were made from the official statistics of the Uppsala County General Sickness Insurance.

Definitions used in acc rdance with the Populati n and Housing Census 1960. According t the definitions given in the housing census for 1960 a *room* is a space measuring at lea t 6 m containing a window and not int nded for the preparation of food. By *household* is meant the person or gr up of pers ns li ing in a certain house or flat. Those defined as *living* in a house or flat are all the persons who for 1961 were registered on the population schedule as being of that address. A household is defined as *overcrowded* if the number living in the house or flat exceeds 2 per room (the kitchen not included).

According t the population census an *income-earning* person is defined as a person who during the so-called population census week, carried out income-earning work during at least half of the normal working time. Also regarded as income-yielding were those persons who had income yielding work but because of temporary discontinuation were not w king during the population census week. This discontinuation was regarded as temporary if its duration was no longe than 4 months. These definitions were used in the present study.

The cla sification of industrial branches was made in accordance with the population census of 1960 and the norms followed were adjusted according t the Indices to the international tandard industrial lassification f all economic activities (ISIC) published in U N s Statistical P pers Ser M No. 4.

VI DESCRIPTION OF CLINICAL COURSE WITH REGARD TO THE ESTIMATED DEGREE OF SEVERITY OF THE BRONCHIAL ASTHMA

The clinical course of the bronchial asthma in this series of patients, with regard to the assessed degree of sever ity of the disease will now be de scribed. I consider such a description to be motivated as a background to the results, which will be given later concerning the long term effect of the disease on the respiratory and circula tory functions and the social adapta tion of the patient.

It was not the aim of the present investiga tion to take into account the extent t which different types of therap had influenced the course of the bronchial sthma in this series. All patients were observed in hospital and treated t least by the prevalent type of ymptomatic therapy The subsequent therapy wa ery irregular It will only be mentioned her that corticosteroids were used practi cally daily for period of at least a month in 19 patients and that specific hyposensiti zation was tried in 44 patients.

T bl 2 shows the variation ranges nd rithmetic mean values for the ge t onset, duration of the disease, number of days of incapacity and symptom days respectively (see page 24) icknes points (see page 25) number of atta ks and total number of attack hours, both totally and di tributed according to age and sex.

The figures in the table, which ap plies to the end of 1961 show that the mean age for both men and women was 54 years. The mean age at onset for the men was 32 years and for the women 33 years, and this increased in direct relationship to age, a higher age group having a higher mean age at onset. This applied to both men and women. The total mean duration up to the end of 1961 was 21 years for both men and women. This duration was practically the same in the different age groups. By total duration is meant that time in years that had elapsed since the onset, minus the number of completely symptom-free calendar years. This involved some uncertainty but for practical reasons this form of assessment was necessary The mean number of completely symptom free calendar years was neg ligible.

The mean number of days of in capacity and symptom days was about 1000 and 2000 respectively for both men and women. The mean total number of sickness points was about 1700 for both men and women. There appeared to be no great difference between the different age groups, but in the women in the younger age group however the mean number of points was relatively low

I 113 of the 116 men and women the

asthma was of the type that involved for the greater part pronounced attacks of symptoms, i e episodic symptoms. For these patients the mean number of attacks was about 540. In the main there was no sex difference in this respect. Among the different age groups it was noted that in the younger men the mean number of attacks was relatively l igh but in the older men relatively low In contrast to the younger men, the younger women had a relatively low mean number of attacks.

The mean number of attack hours was about 3900 for the men and about 3300 for the women The highest number was noted for the younger men, while the lowest was found in the group of younger women. In this respect, as for all the studied factors mentioned above the ranges within the sex and age groups were very large

Case history factor groups acc. to sex and yr of birth		No	range	mean
Age yrs				
Total	M and F	116	29—68	54
	M	42	32—68	54
	F	74	29—08	54
M born	1912—1932	13	32—49	39
„ „	1902—1911	13	50—59	55
„ „	1893—1901	16	60—68	64
F	1913—193?	29	20—49	42
„ „	190?—1911	17	50—59	55
„	1803—1001	28	60—08	65
Age at onset yrs				
Total	M and F	116	2—64	33
	M	42	?—64	32
„	F	74	2—50	33
M born	1912—1032	13	2—41	17
„	1902—1911	13	4—49	3?
„	1893—1901	16	4—64	44
F „	1012—1032	?9	2—42	22
	1902—1911	17	20—51	36
„ „	1803—1901	28	12—50	41
Duration of di ease yrs.				
Total	M and F	116	1—54	21
„	M	42	1—50	21
	F	74	1—54	21
M born	1912—1932	13	1—37	21
	1902 -1911	13	4—50	23
	1803—1901	16	4—41	28
F	1012 -1932	20	2—47	19
	1002—1911	17	1—32	19
„	1803- 1901	28	5—54	23
Days of incapa ity				
Total	M and F	116	0—4010	1008
	M	42	6—4000	960
	F	74	0—4010	1035
M born	1912—1932	13	6—2550	973
	1902—1911	13	14—4000	827
	1803—1901	16	150—3200	1057
F „	1912—1932	29	0—2430	877
	1902—1911	17	45—4910	1147
	1893—1901	28	120—3300	1130

Case history factor groups acc. to sex and yr of birth		No.	range	mean
Symptom days				
Total	M and F	116	76—11460	2080
	M	42	76—11460	2168
	F	74	105— 7680	2030
M born	1012—1032	13	484— 8360	1804
„ „	1902—1911	13	315—11460	2639
„	1893—1901	16	76— 5940	2037
F „	1912—1932	29	210— 3570	1565
	1902—1911	17	105— 4950	2036
	1893—1901	28	222— 7680	2313
Sickness points				
Total	M and F	116	79—7781	1696
	M	42	79—7781	1681
	F	74	79—6553	1?04
M born	1912—1932	13	79—3347	1590
„	1902—1911	13	198—7781	1714
	1893—1901	16	175—4309	1728
F „	1912—1932	29	169—3381	1302
	1902—1911	17	70—6553	1818
„	1893—1901	28	193—4653	1959
No of attacks				
Total	M and G	113	1—4320	556
	M	40	1—4320	537
	F	73	1—2720	567
M born	1912—1932	12	1—4320	607
	1902—1911	13	1—2900	517
	1893—1901	15	20—1600	427
F	1912—1932	29	1—1900	424
	1902—1911	17	1—2720	750
	1893—1901	27	1—3115	606
Attack hours				
Total	M and F	113	8—34080	3640
„	M	40	8—34080	3874
	F	73	8—23234	3320
M born	1012—1932	12	8—34080	5120
	1902—1911	13	8—22680	3120
	1893—1901	13	40—11840	3552
F „	1912—1932	29	8— 6640	2166
	1902—1011	17	16—18218	4066
„	1893—1901	27	16—23234	4252

Tabl 2. *Mean values and ranges for characteristics of the material at the end of 1961 with regard to sex and age and some case history data*

three patients wh se asthma was not of the attack type are eluded f om the Table.

Table 3 divides the age and sex groups into groups according to the estimated total number of sickness points. Group A consists of patients who did not attain 1000 points, group B those who attained 1000 but not 2000 and group C those who attained 2000 sickness points or more. For those belonging to group A the mean number of sickness points was 5 3 for those in group B 1470 and for those in group C 3080 Of the patients in group A, two had fewer than 100 points. On the whole there was even distribution between the groups for both men and women.

Y of birth	MALE A <1000 p	MALE B 1000—1999 p	MALE C ≧2000 p	A+B+C	FEMALE A <1000 p	FEMALE B 1000—1099 p	FEMALE C ≧2000 p	A+B+C
1912—1933	3	6	5	14	12	11	6	20
1902—1911	7	2	4	13	8	7(8)	5(4)	17
1809—1901	2	8	5	15	9	7(8)	12(11)	28
T tal	12	16	14	42	28	25	23	74

T ble 3 *The seri distributed according t sickness point (classes A, B and C), year f birth and sex Sickness point valid at the end of 1961 (in parenthesis the correspond ng distribution 1900 wher th differs from the distribution 1951)*

What form has the asthma course taken since its onset? Has the disease tended towards deterioration or im provement or has there been no noteworthy change? When during the present investigation these questions were put to each of the patients, the great majority about 80 %, stated that on th whole the disease had shown improvement. Only a small number about 5 % considered that their sthma had become worse during the years since its onset. This period, how ever varied greatly among the pa tients, and it was therefore found that detailed analysis on the basis of these norms would not satisfactorily illustrate the course of the asthma in this series. It was decided instead to lassify the patients both according to their age at the time of the investigation and their age at the onset of the dise e and to study for each patient the average number of annual sickness points for certain periods as given below

Points group comprised those patients who during the period in question ttained on an average fewer than 50 sicknes points per year group b those who attained 50 but not 100 point and group those who ttained 100 points or more The following comparison were made

1 Comparison between the sickness point for the first 5 years after onset nd those for the 5-year period almost immediately preceding the time of the present investigation, i. e. the years 1957–1961

2. Comparison between the sickness point for the period 1947 1951 and those for the years 1957 1961.

3. Comparison between the sickness points for the period from the onset of the asthma up to 1957 and those for the period 1957–1961

Patients with an observation period of les than 10 years were excluded from these comparisons.

For the patients who during a certain period had belonged to the groups with the lowest and highest average annual sickness points, re-

Time period and population studied	Year of birth														
	1893—1911									1912—1932					
	Age at onset														
	≦16			17—39			≧40			≦16			17—30		
	Sickness points group														
	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c
Distribution of patients into severity groups during the first 5 years after the onset	1	3	1	ɔ	14	10	7	15	10	7	8	2	6	7	7
Corresponding distribution during the period 1937—61	3	1	1	13	0	8	20	0	3	12	3	2	15	4	1
No. of patients who during the first 5 years after the onset had *slight* asthma.	1			3			7			7			6		
Their distribution into groups by degree of severity during the period 1937—61	1			3	2		5	2		6	1		4	1	1
No. of patients who during the first 5 years after the onset had *severe* asthma			1			10			10			2			7
Their distribution into groups by degree of severity during the period 1937—61		1		3	3	4		2	1			2	6	1	
No. of patients who during the period 1937—61 had "*slight*" asthma.	3			15			20			12			15		
Their distribution into groups by degree of severity during the first 5 years after the onset.	1	2		3	0	3	5	8	7	6	0		4	5	6
No. of patients who during the period 1937—61 had "*severe*" asthma.			1			8			3			2			1
Their distribution into groups by degree of severity during the first 5 years after the onset.		1			4	4		2	1			2	1		

Table 4 *Comparison between the distribution of the patients at two different periods. Grouping according to the degree of severity of the asthma*

The Table shows classified by year of birth and age at onset of the asthma, the number of patients in three severity groups ("slight asthma"=a, moderately severe =b, and severe =c) according to sickness points. A comparison being made between the first 5 years after the onset of the asthma and the period 1937—61. The Table also shows the distribution into severity groups during 1937—61 of those who during the first 5 years after the onset belonged to the groups with the lowest and highest mean annual sickness points, respectively. Also shown in the Table is the distribution into severity groups, during the first 5 years after the onset of those who during 1937—61 belonged to the groups with the lowest and highest mean annual sickness points, respectively.

spectively the distribution between the different severity groups during an earlier or later period of time was studied. It was thus possible not only to study the distribution according to severity within the group but also the development in the group from one period to another.

As shown in Tables 4 and 5 there was an over-all impression of improvement regardless of whether the observation times comprised the first 5 years after the onset and the period 1957–1961 or the two periods 1947–1951 and 1957–1961. Division into groups according to age and age at onset showed that this tendency was common to all groups. The same impression was also obtained on comparison of the sickness point groups for the whole period between the onset up to the end of 1956 with the corresponding groups for the period 1957–1961 (see Table 6).

Time period and population studied	Year of birth														
	1893—1911									1912—1932					
	Age at onset														
	≦16			17—39			≧40			≦16			17—39		
	Sickness points group														
		b			b			b			b			b	
Distribution of patients into severity groups during the period 1947—51	1	3	1	5	10	14	4	8	12	4	8	5	2	8	4
Corresponding distribution during the period 1957—61	3	1	1	15	6	8	15	7	2	12	3	2	10	3	1
No. of patients who during the period 1947—1951 had "slight" asthma	1			5			4			4			2		
Their distribution into groups by degree of severity during the period 1957—61	1			4	1		3	1		4			2		
No. of patients who during the period 1947—51 had "severe" asthma			1			14			12			5			4
Their distribution into groups by degree of severity during the period 1957—61		1		5	3	6	8	2	2	2	1	2	3		1

Table 5. Comparison between distributions of the patients at two different periods. Grouping according to the degree of severity of the asthma.

Classifications as in Table 4. Comparison is made between the period 1947—51 and 1957—61. The Table also shows the distribution into severity groups during 1957—61 of those who during the period 1947—51 belonged to the groups with the lowest and highest mean annual sickness points, respectively.

Comparison between Tables 4 and 5 appears to indicate that the situation in 1957–1961 was somewhat worse than during the first five years after the onset. This may possibly be explained by the selection principles—*those who were admitted to hospital in a period which constituted part of the qualification time belonged relatively often to a group with a high mean number of sickness points for this period.*

On studying the development in those patients (26 altogether) who during the first

Time period and population studied	Year of birth														
	1893—1911									1912—1932					
	Age at onset														
	≦16			17—39			≧40			≦16			17—39		
	Sickness points group														
	a	*b*	*c*	*a*	*b*	*c*	*a*	*b*	*c*	*a*	*b*	*c*	*a*	*b*	*c*
Distribution of patients into severity groups during the total duration up to and including 1956.	3	1	1	4	15	10	6	15	11	4	11	2	4	9	7
Corresponding distribution during the period 1957—61	3	1	1	15	6	8	20	9	3	12	3	2	15	4	1
No of patients who during the total duration up to and including 1956 had *slight* asthma.	3			4			6			4			4		
Their distribution into groups by degree of severity during the period 1957—61	3			3	1		5	1		4			2	2	
No of patients who during the total duration up to and including 1956 had *severe* asthma			1			10			11			2			7
Their distribution into groups by degree of severity during the period 1957—61		1		3	2	5	6	3	2		1	1	5	1	1

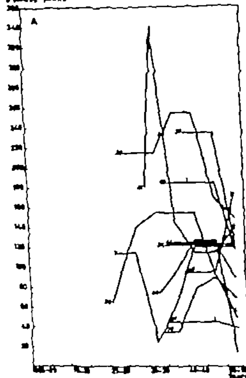
Table 6. *Comparison between distribution of the patients at two different periods. Grouping according to the degree of severity of the asthma.*

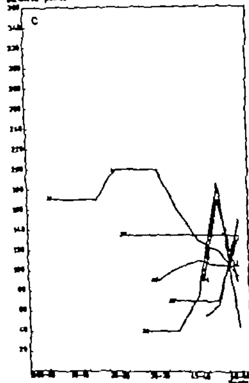
Classifications as in Table 4. Comparison is made between the period which had elapsed from the onset of the asthma up to 1957 and the period 1957—61. The Table also shows the *distribution into severity groups,* during 1957—61 of those who during the period from the onset of the asthma up to 1957 belonged to the groups with the lowest and highest mean annual sickness points respectively.

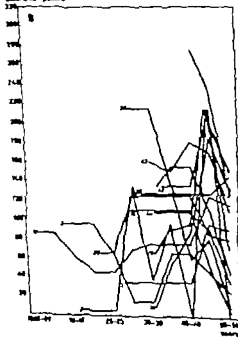
Fig. 1. Changes in the estimated degree of severity with regard to the mean annual number of sickness points for 5-years periods since the onset of the asthma for patients in certain selected groups.

The figure at the start of curve line denotes age at onset. The arrows indicate the years during which invalid pension was received.

Fig. 1 A shows the course for invalid pensioned women, Fig. 1 B for women belonging to the group with ≧ 2000 sickness points (excluding the invalid pensioned). Fig. 1 C for invalid pensioned men, and Fig. 1 D for men belonging to the group with ≧ 2000 sickness points (excluding the invalid-pensioned).







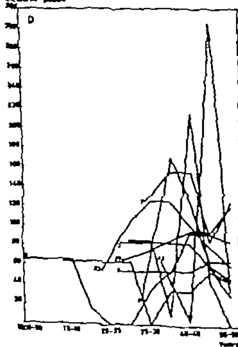


Fig 1

Comparison between Tables 4 and 5 *appears to indicate that the situation in 1017–1931* was somewhat worse than during the first five years after the onset. This may possibly be explained by the selection principles—those who were admitted to hospital in a period which constituted part of the qualification time belonged relatively often to a group with a high mean number of sickness points for this period.

On studying the development *in those* patients (20 altogether) who during the first

Time period and population studied	Year of birth														
	1893—1911									1912—1932					
	Age at onset														
	≦16			17—39			≧40			≦16			17—39		
	Sickness points group														
	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c
Distribution of patients into severity groups during the total duration up to and including 1956.	3	1	1	4	15	10	0	15	11	4	11	2	4	9	7
Corresponding distribution during the period 1957—61	3	1	1	15	0	8	20	0	3	12	3	2	15	4	1
No of patients who during the *total duration* up to and including 1956 had *slight* asthma.	3			4			0			4			4		
Their distribution into *groups* by degree of severity during the period 1957—61	3			3	1		5	1		4			2	2	
No of patients who during the total duration up to and including *1956 had severe* asthma.			*1*			*10*			*11*			2			7
Their distribution into groups by degree of severity during the period 1957—61		1		3	2	5	6	3	2		1	1	5	1	1

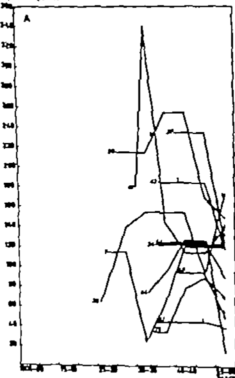
Table 6. *Comparison between distribution of the patients at two different periods. Grouping according to the degree of severity of the asthma.*

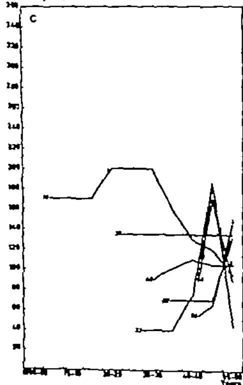
Classifications as in Table 4 Comparison is made between the period which had elapsed from the onset of the asthma up to 1957 and the period 1957—61 The Table also shows the distribution into severity groups, during 1957—61 *of those who during the period from the* onset of the asthma up to 1957 belonged to the groups with the lowest and highest mean *annual sickness points, respectively*

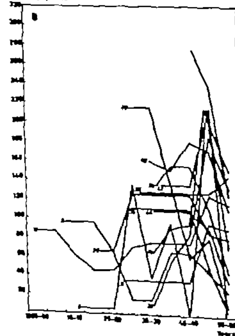
Fig 1 Change in the estimated degree of severity with regard to the mean annual number of sickness points for 5-years periods since the onset of the asthma for patients in certain elected groups

The figure at the start of curve line denotes age at onset. The arrows *indicate the years* during which invalid pension was received.

Fig. 1 A shows the course for invalid pensioned women, Fig 1 B *for women belonging* to the group with ≧ 2000 sickness points (excluding the invalid pensioned) Fig 1 C for invalid pensioned men and Fig 1 D for men belonging to the group with ≧ *2000 sickness points* (excluding the invalid-pensioned)







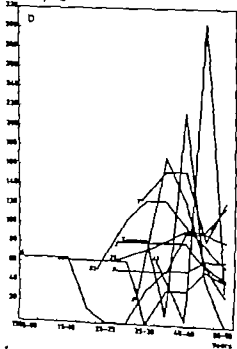


Fig 1

five years after the onset belonged to group a, it was found that only one was assigned to group c during the period 19 7–1961 i e. only one showed marked deterioration (see Table 4) A corresponding study of those who belonged to group c from the beginning (30 patients) showed that 16 of these were to be found in group a in the period 1957–1961 while 7 remained in group c Table 4 suggests that the development in the patient with an onset on or before the age of 16 years does not appear to be so favourable as that in the patients with a later onset age

Table 4 shows *further that of those in* whom the onset occurred on or before the age of 16 years and who in 1957–1961 belonged *to group a not one belonged to group c* during the first five years after the onset and that none of those who were in group c *in 19 –1961 belonged to group a during the* first 5 years after the onset of the disease Of the 15 patients—regardless of age or age at the onset of the asthma—who belonged to group c in 1957–1961 there was only one in group a during the first 5 years after the onset

Thus for the patients in this material there appeared to be some relationship between the degree of severity of the asthma during the first 5 years after *the onset and that during the period* 1957–1961 this relationship appeared *to be more* striking in those in whom the asthma was first manifested at or before the age of 16 years.

The picture of the course of the *bronchial asthma* with regard to its assessed degree of severity shown in Tables 4—6 is complemented by Fig 1 This shows the course from the time of onset up to the time of the *present* investigation for each of the patients who received an invalid pension solely or mainly by reason of bronchial asthma and also for those who according to the *group* classification in Table 3 belonged to group C, i e those who during the period since the onset of *the asthma had attained 2000* sickness points or more.

As the figure shows, the trend varied for different patients and the intensity of the disease in one and the same patient often varied greatly in *different periods It is also shown that* a favourable development with regard to the intensity of the disease in the majority of patients is characteristic of the years immediately preceding the time *of the present investigation.*

In this description no consideration was taken of the extent to which different forms of therapy had influenced the course *It seemed obvious how ever that the favourable development* with respect to the patient s symptoms was greatly due to the therapeutic advances made in recent years. But it is also clear that in many patients *the disease showed a spontaneous* tendency towards improvement The tables and figures show that it was essentially during the years preceding the time *of this investigation that the improvement* took place.

Relationship between pairs of case history *factors*

In order to illustrate the relationship between *different case history factors*, a regression analysis was performed This comprised all of the eight factors that are given in the quantification description *below Table 7 shows the relationship* between pairs of case history factors expressed both by corre

lation coefficients and by significance asterisks.

Between, for example, the variable for the total number of ickness points nd that for the number of admissions t hospital, there was positive correlation coefficient of 0.21. This meant that for the patients with high t tal number of ickness point d mission t hospital was mor common than for patient with low number

Between the riable for the total umber of sicknes point nd that for the ag at onset there w negative correlation of −0.22. Thi meant that the mean ge t onset wa lower for patients with high total number of sicknes point tha for patients with low umber

The different case history factors are quantified as below:

X_1 is the sex variable

$X_1 = 0$ for women		(74 patient)
$X_1 = 1$ for me		(42)

X_2 is the age variable

$X_2 = 1$ for patient	60—68 years old	(41 patient)
$X_2 = 2$	50—59	(30)
$X_2 = 3$	30—49	(42)

X_3 is the variable for the total umber of sickness points

$X_3 = 1$ for <	1000 point	(38 patient)
$X_3 = 2$	1000—1999	(41)
$X_3 = 3$	≥ 2000	(37)

X_4 i the variable for duration

$X_4 = 1$ for <	15 years	(32 patient)
$X_4 = 2$	15—24	(31)
$X_4 = 3$	≥ 25	(33)

X_5 i the variable for age t onset

$X_5 = 1$ for ≤	16 years	22 patients)
$X_5 = 2$	17—39	(31)
$X_5 = 3$	≥ 40	(43)

X_6 is the variable for the number of attack hours

$X_6 = 1$ for	1—499 hours	(37 patients)
$X_6 = 2$	500—2499	(23)
$X_6 = 3$	≥ 2500	(44)

X_7 is the ariable for the mean number of sickness point per year during the period 1957—1981

$X_7 = 1$ for <	50 poi t	(0 patients)
$X_7 = 2$	50—99	27)
$X_7 = 3$	≥ 100	(18)

X_8 the variable for the number of times admitted to hospital

$X_8 = 1$ for	1—4 admission	(81 patient)
$X_8 = 2$	5—9	(21)
$X_8 = 3$	≥10	(14)

Case history factor		X_1	X	X_3	X_4	X_5	X_6	X_7	X_8
Sex	X_1		−0.05	0.05	0.02	−0.07	0.01	0.06	0.08
Age	X_2	−0.05		−0.12	−0.08	−0.58**	0.04	−0.10	−0.15
Total sickness points	X_3	0.05	−0.12		0.59***	−0.32***	0.60***	0.32***	0.31***
Duration	X_4	0.02	−0.08	0.59***		−0.51***	0.37**	0.13	0.17
Age at onset	X_5	−0.07	−0.58***	−0.32***	−0.51**		−0.27**	−0.01	−0.04
Attack hours	X_6	0.01	0.04	0.60***	0.27**	−0.27*		0.20	0.35***
Sickness points 1957—61	X_7	0.06	−0.10	0.32*	0.13	−0.01	0.20		0.31**
Admissions to hospital	X_8	0.08	−0.15	0.31***	0.17	−0.04	0.35***	0.34***	

Table 7 *Correlation coefficients — with significance asterisks — between pairs of case history factors*

X_1—X_8 refer to the different case history factors.

Significance test as in point 7 page 159

The table shows that there is a highly significant relationship between different pairs of case history factors. As might be expected, it is found that the total number of sickness points is the factor that, paired with others, shows the highest number of significant relationships.

The *sex* factor shows only insignificant relationships with pairs of other factors.

There is a significant relationship between the *age* factor and the age at *onset* due to the fact that in this series the patients with a relatively high mean age also had a relatively high *age at onset*.

The *total number of sickness points* is highly significantly correlated to the *duration*, *age at onset*, *attack hours* and *sickness points during* the five-year period almost immediately preceding the time of the investigations, i. e. 1957–61 and to the number of hospital admissions on account of bronchial asthma. This means that on an average the patients with a high total number of sickness points also had a long duration, a low age at onset, a high number of attack hours, a high annual number of sickness points during the years 1957–61 and a high number of admissions to hospital.

The *duration* is highly significantly correlated to the age at onset, and significantly correlated to the number of attack hours. This means that patients with a long mean duration had an early age at onset and a high number of attack hours.

The *age at onset* is significantly correlated to the number of attack hours, patients with a high age at onset having on an average a low number of attack hours.

The number of *attack hours* is highly significantly correlated to the number of admissions to hospital, patients with a high number of attack hours having on an average a high number of admissions to hospital.

The *number of sickness points during the period 1957—61* is highly significantly correlated to the number of admissions to hospital, patients with a high number of sickness points during this period having on an average a high number of admissions to hospital during the whole period since the onset of asthma

---

Since the total number of sickness points shows a highly significant correlation to all the other factors that are characteristic of the case history of an asthma patient, the grouping of the patients in the main part of the subsequent analysis of the results will be based on the total number of sickness points.

VII RESPIRATORY AND CIRCULATORY FUNCTION IN RELATION TO THE CLINICAL COURSE AND ESTIMATED DEGREE OF SEVERITY OF THE BRONCHIAL ASTHMA

The results of the investigations on the effects of bronchial asthma on respiratory and circulatory function in the patients in this *series are in principle* divided into three sections Because of the close relationship between the cardiovascular and respiratory functions however no sharp line of demarcation is drawn between them The first section treats ventilation capacity and lung volumes, the second physical work capacity and some aspects of its relation to lung function and the third section hemodynamics and some aspects of its relation to lung function.

Those patients who had or had had anoth r chronic disease — in addition to the bronchial asthma — whicl might have had a d termin i g influence on th respiratory nd circula l *ry functions*, are n t I luded in the general analyses of the results, r the discu sions. Th result obtained from nvestigations n these patient are reported separat ly when con sid red of interest

Before eporting the respiratory and cir ulatory data obtained it should be emphasi zed *that in the patient to whom these data* apply no abnormal ymptoms of cardiac decompensation or coronary in uffi iency w r pres nt at th time f the present investigation. *No definit* signs of hypertrophy o incompensation of the l ft or right en tricle were found either on physical or roentgenologic *xamination or on ECG* N ither w re there a y definit signs of alvular or *coronary heart disease* in the patient included in the general analyses. As m nti ned previously a separate report is made on those patients in whom abnormal conditions such *as these* w re *bserved this* category also includes patients with more *pronounced* pulmonary changes due e g to tuberculosis, seen roentgenologically and also those who *had or had had chronic bronchitis. The num*ber of patients with signs and symptoms of a noteworthy type was 12 The case histories of these patients as for those in whom there *were signs of an additional disease* — not however assessed as being of decisive importance — are given on pages 160–164

In Table 8 some anthropometric data are given for the patients in the asthma series, grouped according to age and sex. Figs. 2 3 and 4 show the relationships between different dimensions in the cardiovascular system with the patients grouped according to their estimated total number of sickness points *(see page 25) The mean cir*culatory *function* capacity (expressed as W_{130}=load in kpm/min at heart rate 130 beats/min) was found to be lower in patients with a high number of sickness points than in those with a low number This difference was *statistically significant. In none of these groups* of patients was the total Hb content (THb) found to vary signifi cantly with the circulatory function capacity This means thus that the ratio

W_{130}/THb was on an average somewhat lower for patients with a high than with a low number of sickness points, but the difference was not, however statistically significant. Neither were the relationships between the total cardiac volume and W_{130} and THb respectively significantly influenced by the number of sickness points.

Sex Age		Height cm	Weight kg	B.S. m²	[illegible] %	Heart vol. ml	THb g	THb g/kg	Hb conc. g/100 ml	Blood vol. l	Blood vol. ml/kg
Male											
20—49	No.	13	13	13	13	13	13	13	13	13	13
	mean	173	72	1.86	+4.6	615	696	9.94	14.70	5.29	74.7
	highest	194	91	2.20	+21	835	952	15.71	16.19	7.53	118.4
	lowest	163	53	1.56	— 6	303	441	6.87	12.74	3.07	49.8
50—59	No.	13	13	13	13	13	12	12	13	12	12
	mean	170	70	1.80	+6.3	610	679	8.32	14.57	4.25	63.5
	highest	184	92	2.13	+26	733	839	11.81	16.41	5.60	87.9
	lowest	154	48	1.53	—11	406	483	5.37	11.70	3.36	42.7
60—68	No.	16	16	16	16	16	16	16	16	16	16
	mean	173	71	1.85	+9.1	631	633	8.80	14.58	4.77	67.7
	highest	181	88	2.06	+35	803	906	12.50	16.96	6.72	90.5
	lowest	161	56	1.68	—10	515	437	6.25	11.95	3.35	48.6
Total	No.	42	42	42	42	40	41	41	41	41	41
	mean	172	71	1.84	+6.6	626	633	9.02	14.62	4.82	69.0
	highest	194	91	2.20	+43	835	952	15.71	16.96	7.53	118.4
	lowest	154	48	1.52	—11	303	403	5.37	11.70	3.07	42.
Female											
20—49	No.	29	29	29	28	28	28	26	28	28	28
	mean	162	61	1.66	+2.9	503	431	6.90	13.16	3.63	58.4
	highest	169	86	1.91	+22	655	610	9.83	14.67	4.05	78.4
	lowest	152	47	1.44	—23	378	303	4.93	10.91	2.73	38.0
50—59	No.	17	17	17	16	17	14	14	14	15	15
	mean	160	64	1.66	+0.3	513	463	7.36	12.93	4.01	63.9
	highest	171	80	1.83	+14	699	609	11.10	14.88	6.00	100.0
	lowest	152	50	1.48	—11	389	312	4.66	10.63	3.30	49.2
60—68	No.	28	28	28	25	27	28	28	28	28	28
	mean	161	66	1.68	+9.5	553	501	7.74	13.66	4.03	62.1
	highest	171	94	2.01	+40	920	836	12.36	15.83	6.60	91.9
	lowest	153	44	1.38	—11	365	299	5.07	11.09	3.90	43.1
Total	No.	74	74	74	70	72	69	66	68	69	69
	mean	161	65	1.66	+4.9	524	467	7.34	13.32	3.89	61.5
	highest	171	94	2.01	+40	920	836	12.36	15.83	6.60	100.0
	lowest	152	44	1.36	—23	365	299	4.66	10.63	2.73	38.0

Table 8 *Mean values, highest and lowest values of some anthropometric data.*

In this and the following tables showing age, in this chapter the ages of the patients refer to 1st Jan 1962.

One woman born in 1932, and who had not quite reached the age of 30 years, is combined in this and the following tables showing age distribution with the 30-year old patients.

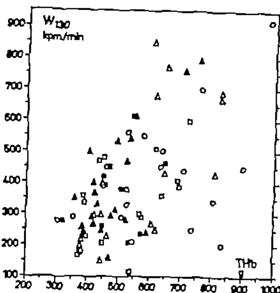


Fig 2 The relationship between rate of wo k at a heart rate of 130 beats/min (W_{130}) kpm/min, and total amount of hemoglobin, g

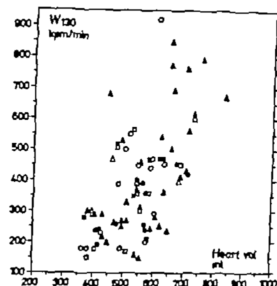


Fig 3 The relationship between rate of work at a heart rate of 130 beats/min (W_{130}) kpm/min and heart volume ml.
Symbols as in Fig. 2.

Triangles represent patients who bel ng to group A (page 35) squares those belonging to group B circles those belonging to group C. *Open symbols represent patients who have* been at a steady state at a heart rate of 130 beats/min, and filled symbols those who have not

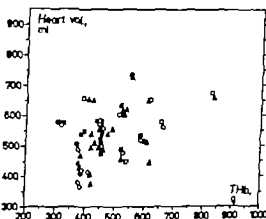


Fig 4 The relation hip between heart volume ml and total hemoglobin content g
Symbols as in Fig. 2.

LUNG FUNCTION STUDIES

Spirometric lung function studies were performed on the whole main series (116 patients) The ventilation capacity and also the lung volumes (with the helium dilution method) were studied. In 7 of the women, how ver the lung volume measurements vere not satisfactory The analyses of he values *obtained for functional* esidual capacity residual volume and total lung capacity were therefore based on only 109 of the 116 patients. 12 patients who were *diagnosed as* having a chronic disease in addition to the bronchial asthma are reported separately (page 63)

Table 9 gives the mean values and ranges (lowest and highest values) of *the data obtained on ventilation capa city and lung volumes.*

Sex age, yrs		No.	VC l	FEV1 l	FEV%	MVV40 l/min	MVVf l/min	No.	TLC l	FRC/TLC	RV/TLC
Male											
60—68	mean	16	2.7	2.1	65	63	73	16	6.8	63	49
	highest		5.1	3.5	74	92	103		10.6	77	66
	lowest		2.0	0.7	28	31	28		4.6	54	40
50—59	mean	13	3.9	2.2	65	64	77	13	6.2	59	47
	highest		4.8	3.1	71	114	121		7.8	74	63
	lowest		3.0	1.2	35	35	43		5.1	43	30
30—49	mean	13	4.9	3.2	63	100	118	13	7.2	65	37
	highest		6.3	5.0	86	163	216		9.3	66	43
	lowest		4.0	1.9	46	46	82		5.8	44	26
Female											
60—68	mean	28	2.5	1.5	60	44	50	28	4.5	63	50
	highest		3.6	2.3	80	66	92		5.8	80	73
	lowest		1.4	0.5	38	22	24		2.8	47	30
50—59	mean	17	2.8	1.7	60	53	62	15	4.4	61	46
	highest		3.9	2.7	86	85	119		5.7	71	66
	lowest		2.0	0.9	43	28	37		3.4	47	30
30—49	mean	20	3.3	2.2	67	68	80	25	5.0	54	39
	highest		3.9	3.3	81	99	124		5.9	73	53
	lowest		2.7	1.1	41	33	35		3.5	37	26

Table 9. *Mean values, highest and lowest values, concerning lung volume and ventilation capacity in different patient groups, classified according to sex and age*

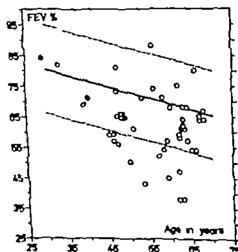


Fig. 5. FEV% in relation to age for the males.

Central line indicates normal regression between these parameters. Interrupted lines indicate ± twice standard error of estimate (Berglund, Birath, Bjure, Grimby, Kjellmer, Sandqvist and Söderholm, 1963)

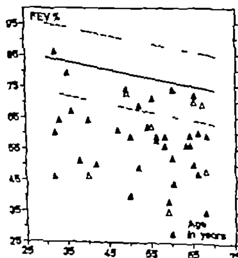


Fig. 6. FEV% in relation to age for the males.

Central line indicates normal regression between these parameters. Interrupted lines as in Figure 5.

	VC	$FEV_{1.0}$	FEV%	MVV_{40}	MVV_f	TLC	FRC/TLC	RV/TLC
VC		0.71***	0.26**	0.64***	0.59***	0.42***	—0.40**	—0.41***
$FEV_{1.0}$	0.71***		0.83 **	0.81 **	0.79***	0.00	—0.48***	—0.52**
FEV%	0.26	0.83**		0.67***	0.66***	—0.24	—0.39***	—0.42***
MVV_{40}	0.64***	0.81	0.67***		0.91***	0.10	—0.43***	—0.40***
MVV_f	0.59***	0.70***	0.60**	0.91 **		0.01	—0.44***	—0.54***
TLC	0.42***	0.00	—0.24	0.10	0.01		0.20*	0.27**
FRC/TLC	—0.40***	—0.48**	—0.39***	—0.43 **	—0.44 **	0.20*		0.77***
RV/TLC	—0.41	—0.52***	—0.42**	—0.40**	—0.54***	0.27**	0.77***	

Table 10 *Correlation coefficients — with significance asterisks — between pairs of indices of respiratory function*
The indices are expressed as ratios between observed and predicted values for each subject.

Figures 5 and 6 illustrate the position of the patients in the asthma series and regression lines for the control series with regard to FEV% and age. It is shown that the majority of the women are found between the two interrupted lines corresponding to regression line ± 2 residual SD while this is not valid for the men.

Table 10 shows the degree of agreement between pairs of different lung function tests. This agreement is expressed by means of correlation coefficients, the significance of which is demonstrated by asterisks. Very strong relationship is seen between the majority of combinations of pairs of lung function tests. The highest correlation coefficient (0.91) is obtained for MVV_{40}—MVV_f. This relationship is illustrated further by the following: for each of these two tests the 20 patients who were recorded as having the least favourable, i. e. the lowest values were selected from the total 116. Each patient was represented by the highest value which he produced and this value is given as a percentage of the predicted value. Of the 20 patients no fewer than 17 were commonly selected for both MVV_{40} and MVV_f. This figure was compared with an expected figure — assuming that there was no relationship — of 3.5. All correlations are significant except between three pairs, i. e. TLC — $FEV_{1.0}$, TLC — MVV_{40} and TLC — MVV_f.

Mean values and ranges for data obtained in different tests of ventilation capacity and lung volume

For each of the lung function tests the mean figures and ranges are given both for the entire material and for the material divided into groups according to sex and assessed degree of severity of the disease based on the total number of sickness points. The results are given both as the difference from the predicted value (mean figure and distribution) and as the mean of the individual ratios between observed and predicted values expressed in per cent. The predicted values are obtained according to Table 1 (for method see page 28).

Vital capacity (VC)

As shown in Table 11 the mean value for the vital capacity expressed as a percentage of the predicted value was 85.0 for the men and 87.1 for the women. The mean difference between the observed and the predicted value was −0.7 litre for the men and −0.4 litre for the women. This difference deviates significantly from zero both for the men and for the women (*).

The patients were classified into groups according to the estimated total number of sickness points since the onset of the asthma, and the quotient between the observed value and the predicted value (expressed in per cent) was obtained for each individual. For those with fewer than 1000 points (group A) this mean percentage value was 89.6. For those who had attained 1000 but not 2000 points (group B) this value was 83.9 %, and for those who had attained 2000 or more (group C) 85.6 %.

The mean difference between group A and groups B and C combined, and also between group B and group C, was then calculated. The difference between A and B+C was significant (*) when the relative frequency of cases with a positive difference by χ^2 test was compared (cf page 159). No significant difference was found between B and C.

Patients according to sex and sickness points	Difference between observed VC and predicted value (litres)										Difference from the predicted value (litres) mean	Percentage of predicted value mean
	No.	≥ +0.7	+0.3 +0.6	+0.2 −0.2	−0.3 −0.6	−0.7 −1.0	−1.1 −1.4	−1.5 −1.8	−1.9 −2.2	≤ −2.3		
Male	42	0	3	9	11	6	7	3	2	1	−0.7***	85.0
Female	74	1	3	20	27	18	4	1	0	0	−0.4***	87.1
Total	116	1	6	29	38	24	11	4	2	1	−0.5***	86.3
Group A												
Male	12	0	1	3	3	2	1	1	0	1	−0.7*	86.1
Female	26	0	0	14	8	3	1	0	0	0	−0.3***	91.2
Total	38	0	1	17	11	5	2	1	0	1	−0.4***	89.6
Group B												
Male	16	0	0	4	3	1	6	1	1	0	−0.9***	82.1
Female	25	0	2	3	9	9	2	0	0	0	−0.5***	85.0
Total	41	0	2	7	12	10	8	1	1	0	−0.7***	83.9
Group C												
Male	14	0	2	2	5	3	0	1	1	0	−0.9*	87.5
Female	23	1	1	3	10	6	1	1	0	0	−0.5***	84.7
Total	37	1	3	5	15	9	1	2	1	0	−0.5***	85.6

Table 11. Vital capacity (VC) in different patient groups, classified according to sex and estimated number of "sickness points" since the manifestation of the bronchial asthma. The differences between the individual values of VC and the individual predicted values (predicted according to sex, age and height) are given. The significance of difference from the predicted value is stated with significance asterisks. Means of the individual ratios between observed and predicted value expressed in per cent are also given.

Forced expiratory volume in one second ($FEV_{1.0}$)

As may be seen in Table 12 the mean value for the forced expiratory volume in one second given as a percentage of the predicted value, was 70.0 for the men and 70.8 for the women The mean difference between the observed value and predicted value was −1.0 litre for the men and −0.7 litre for the women This difference deviates significantly from zero for both the men and the women (***)

The patients were classified into groups according to the estimated total number of sickness points since the onset of the asthma and the quotient between the observed and the predicted value (expressed in per cent) was obtained for each individual For those with fewer than 1000 points (group A) this mean percentage value was 78.6. For those who had attained 1000 but not 2000 points (group B) a value of 67.5 % was obtained, and for those who had attained 2000 points or more (group C) 65.5 %

The mean difference between group A and groups B and C combined, and also between group B and group C, were then calculated. The difference between A and B+C was found to be significant (**) There was no significant difference between B and C. (Significance test according to point 2 on page 159)

Patients according to sex and sickness points	Difference between observed $FEV_{1.0}$ and predicted value (litres)										Difference from the predicted value (litres) mean	Percentage of predicted value mean
	No	≥ +0.7	+0.3 +0.6	+0.2 [illegible]	−0.3 −0.6	−0.7 −1.0	−1.1 −1.4	−1.5 −1.8	−1.9 −2.2	≤ −2.3		
Male	42	1	0	5	8	12	5	4	4	3	−1.0***	70.0
Female	74	0	0	9	21	25	16	3	0	0	−0.7**	70.8
Total	*116*	*1*	*0*	14	29	37	21	7	4	3	−0.9***	70.5
Group A												
Male	*12*	*1*	*0*	3	2	2	1	1	1	1	−0.8*	77.3
Female	20	0	0	5	11	9	1	0	0	0	−0.5***	79.2
Total	38	1	0	8	13	11	2	1	1	1	−0.6***	78.6
Group B												
Male	10	0	0	1	3	6	1	3	1	1	−1.1***	6 .3
Female	25	0	0	2	5	9	7	2	0	0	−0.9***	67.6
Total	41	0	0	3	8	15	8	5	1	1	−1.0***	6 .5
Group C												
Male	14	0	0	1	3	4	3	0	2	1	−1.1***	66.9
Female	23	0	0	2	5	7	8	1	0	0	−0.9*	64.7
Total	37	0	0	3	8	11	11	1	2	1	−1.0***	65.5

Table 12 *Fo d expiratory volume in one second ($FEV_{1.0}$) in different patient gro ps classified according to sex and estimated number of "si kness points" since the manifest ti n of the bronchial asthma. The differences between th individual value $FEV_{1.0}$ and the individual predicted values (prediction according to sex, age and height) are given. The significance of difference from the predicted value is stated with significance asterisks. Means of the individual rati between observed and predicted val es expre ed in per cent are also given*

Forced expiratory volume in one second ($FEV_{1.0}$) expressed as a percentage of vital capacity (VC) i.e. FEV%

Table 13 shows that the mean value for the forced expiratory volume in one second ($FEV_{1.0}$) expressed as a percentage of the vital capacity (FEV%) was 79.8 % of the predicted value for the men and 79.6 % for the women. The mean difference between the observed and the predicted value was −14.2 per cent units for the men and −15.9 per cent units for the women. This difference deviated significantly from zero for both the men and the women ( )

The patients were classified into groups according to the estimated total number of sickness points, and the quotient between the observed and the predicted value (expressed in per cent) was obtained for each patient. For those with fewer than 1000 sickness points (group A) this mean percentage value was 86.3 For those who had attained 1000 but not 2000 points (group B) a value of 78.0 % was obtained, and for those who had attained 2000 points or more (group C) 74.7 %

The mean difference between group A and groups B and C combined, and also between group B and group C, were than calculated. The difference between A and B+C was found to be significant (*) No significant difference was found between groups B and C.

Patients according to sex and sickness points	Difference between observed FEV% and predicted value (per cent units)										Difference from the predicted value (per cent units) mean	Percentage of predicted value mean
	No.	≥ +11	+10 +6	+5 −5	−6 −10	−11 −15	−16 −20	−21 −25	−26 −30	≤ −31		
Male	42	0	1	9	5	11	5	2	3	6	−14.2***	79.8
Female	74	0	1	11	15	14	10	9	5	9	−15.9***	79.6
Total	116	0	2	20	20	25	15	11	8	15	−15.3***	79.7
Group A												
Male	12	0	0	6	1	2	0	1	1	1	−8.8*	86.7
Female	26	0	1	6	5	5	6	3	0	0	−10.8***	86.1
Total	38	0	1	12	6	7	6	4	1	1	−10.3***	86.3
Group B												
Male	16	0	1	2	4	2	3	1	2	1	−14.2**	79.8
Female	25	0	0	2	5	5	3	4	4	2	−18.1***	76.9
Total	41	0	1	4	9	7	6	5	6	3	−16.6***	78.0
Group C												
Male	14	0	0	1	0	7	2	0	0	4	−18.1***	74.0
Female	23	0	0	3	5	4	1	2	1	7	−19.2***	75.1
Total	37	0	0	4	5	11	3	2	1	11	−18.8***	74.7

Table 13 *Forced expiratory volume in one second ($FEV_{1.0}$) expressed as percentage of vital capacity (VC), FEV%, in different patient groups, classified according to sex and estimated number of sickness points since the manifestation of the bronchial asthma. The differences between the individual values of FEV% and the individual predicted values (prediction according to sex and age) are given. The significance of difference from the predicted value is stated with significance asterisks. Means of the individual values between observed and predicted values, expressed in per cent are also given.*

Maximum voluntary ventilation deter mined at a fixed frequency of 40 breaths per minute (MVV_{40})

As may be seen in Table 14 the mean value for the maximal voluntary ventilation at a fixed frequency of 40 breaths per minute (MVV_{40}) was 60.3 % of the predicted value for the men and 61 3 % for the women The mean difference between the observed and the predicted value was –48 0 litres for the men and –33 0 litres for the women This difference deviated signi ficantly from zero both for the men and the women (***)

The patients were classified into *groups according to the estimated total* number of sickness points and the quotient between the observed and the predicted value (expressed in per cent) was obtained for each patient. For those with fewer than 1000 points (group A) this mean value was 68 4 %. For those who had attained 1000 but not 2000 points (group B) a value of 56 7 % was obtained, and for those who had attained 2000 points or more (group C) 57 9 %

The mean difference between group A and groups B and C combined, and also between group B and group C, were then calculated. The difference between A and B+C was found to be significant (**) No significant differ *ence was found between B and C.*

I alle ts according to sex and sickness points	Difference between observed MVV40 and predicted value (litres)										Differ ence from the predicted value (litres) mea	Percent age of predicted alue mean
	No	≥ + 1	+20 +11	+10 −10	−11 −20	−21 −30	−31 −40	−41 −50	−51 −60	≤ −61		
Mal	12	0	0	3	3	5	3	7	0	12	—48 0***	60.3
Femal	4	*0*	*0*	*6*	*11*	*12*	20	*18*	*6*	1	—33.0***	61.3
Total	116	0	0	9	14	17	23	25	15	13	—38.8***	00.0
Group A												
Male	12	0	0	3	2	0	0	3	1	3	—37 7**	69.2
F male	26	0	0	4	5	4	8	5	0	0	—28.0***	68 0
Total	38	0	0	7	7	4	8	8	1	3	—31 1***	68.4
Group B												
M le	10	0	0	0	1	1	2	3	5	4	—53.5**	54.9
Femal	[illegible]	0	0	0	4	3	8	7	3	0	—37.0***	57.8
T t l	41	0	0	0	5	4	10	10	8	4	—43 4**	56 7
Group C												
Mal	14	0	0	0	0	*4*	*1*	*1*	*3*	*5*	—50 6***	58 7
F male	23	0	0	2	2	5	4	6	3	1	—36.4***	57 4
Total	37	0	0	2	2	9	5	7	6	6	—41 8***	9

Table 14 *Maxim voluntary ventilation dete mi ed at a fixed frequency of 40 breaths per minute (MVV_{40}) in different patient groups classified according to sex and estimated number of sicknes oints" sinc the manifestat on of the bronchial asthma. The difference bet we n the ind idual value of MVV_{40} and the individual predicted values (predicted according to s x and age) are given Th significance of differ nce from the predicted value is t ted with significance asterisks Means of the individual ratios between observed and predicted valu s expressed in per cent are al given*

Maximum voluntary ventilation determined at a frequency chosen by the patient (MVV$_f$)

As may be seen in Table 15 the mean value for the maximum voluntary ventilation determined at a frequency chosen by the patient was 68.0 % of the predicted value for the men and 59.8 % for the women. The mean difference between the observed and the predicted value was −62.0 litres for the men and −42 0 litres for the women. This difference deviated significantly from zero both for the men and for the women (**).

The patients were classified into groups according to the estimated total number of sickness points since the onset of the asthma, and the quotient between the observed and the predicted value (expressed in per cent) was obtained for each patient. For those with fewer than 1000 points (group A) this mean value was 68 1 %. For those who had attained 1000 but not 2000 points (group B) a value of 54 1 % was obtained, and for those who had attained 2000 points or more (group C) 55 6 %.

The mean difference between group A and groups B and C combined, and also between group B and group C, were then calculated. The difference between A and B+C was found to be significant (**). No significant difference was found between B and C.

Patients according to sex and sickness points	Difference between observed MVV$_f$ and predicted value (litres)										Difference from the predicted value (litres) mean	Percentage of predicted value mean
	No.	≥ +21	+20 +11	+10 −10	−11 −20	−21 −30	−31 −40	−41 −50	−51 −60	≤ −61		
Male	42	1	0	0	0	4	8	4	4	23	−62.0***	68.0
Female	74	0	1	5	3	10	11	13	17	13	−42.0***	59.8
Total	116	1	1	5	3	14	17	17	21	35	−49.2***	60.3
Group A												
Male	12	1	0	0	0	2	2	1	1	5	−48.5**	67.3
Female	26	0	1	3	3	4	5	4	5	1	−33.0***	68.5
Total	38	1	1	3	3	6	7	5	6	6	−37.9***	68.1
Group B												
Male	16	0	0	0	0	1	2	2	0	11	−60.4***	52.6
Female	25	0	0	0	1	4	2	7	5	6	−47.8***	55.0
Total	41	0	0	0	1	5	4	9	5	17	−53.2***	54.1
Group C												
Male	14	0	0	0	0	1	2	1	3	7	−63.2***	56.3
Female	23	0	0	2	1	2	4	2	7	5	−46.0***	55.9
Total	37	0	0	2	1	3	6	3	10	12	−53.3***	55.6

Table 15. Maximum voluntary ventilation determined at a frequency chosen by the patient (MVV$_f$) in different patient groups classified according to sex and estimated number of "sickness points" since the manifestation of the bronchial asthma. The difference between the individual value of MVV$_f$ and the individual predicted values (prediction according to sex, age and height) are given. The significance of difference from the predicted values is stated with significance asterisks. Means of the individual ratios between observed and predicted values expressed in per cent, are also given.

Maximum voluntary ventilation determined at a fixed frequency of 40 breaths per minute (MVV_{40})

As may be seen in Table 14 the mean value for the maximal voluntary ventilation at a fixed frequency of 40 breaths per minute (MVV_{40}) was 60.3 % of the predicted value for the men and 61.3 % for the women The mean difference between the observed and the predicted value was −48.0 litres for the men and −33 6 litres for the women This difference deviated significantly from zero both for the men and the women (***)

The patients were classified into groups according to the estimated total number of sickness points, and the quotient between the observed and the predicted value (expressed in per cent) was obtained for each patient For those with fewer than 1000 points (group A) this mean value was 68.4 %. For those who had attained 1000 but not 2000 points (group B) a value of 56.7 % was obtained, and for those who had attained 2000 points or more (group C) 57 9 %.

The mean difference between group A and groups B and C combined, and also between group B and group C, were then calculated. The difference between A and B+C was found to be significant (**) No significant difference was found between B and C.

Patients according to sex and sickness points	Difference between observed MVV_{40} and predicted valu (litres)										Difference from the predicted value (litres) mean	Percentage of predicted alue mean
	No	≥ + 1	+ 90 +11	+10 −10	−11 −20	−21 −30	−31 −40	−41 −50	−51 −00	≤ −61		
Male	42	0	0	3	3	5	3	7	8	12	−48.0***	60 3
Female	74	0	0	6	11	12	20	18	6	1	−33.6***	61.3
Total	116	0	0	9	14	17	23	25	15	13	−38.8***	60.9
Group A												
Male	12	0	0	3	2	0	0	3	1	3	−37 7**	69 2
Female	26	0	0	4	5	4	8	5	0	0	−28 0***	68 0
Total	38	0	0	7	7	4	8	8	1	3	−31 1***	68.4
Group B												
Male	16	0	0	0	1	1	2	3	5	4	−53 5**	54.9
Female	2›	0	0	0	4	3	8	7	3	0	−37.0***	57.8
Total	41	0	0	0	5	4	10	10	8	4	−43 4***	56.
Group C												
Male	14	0	0	0	0	4	1	1	3	5	−50.6**	58.7
Female	23	0	0	2	2	5	4	6	3	1	−36.4 **	57 4
Total	37	0	0		2	9	5	7	6	6	−41.8***	57 9

Table 14 *Maximum voluntary ventilation determined at a fixed frequency of 40 breaths per minute (MVV_{40}) in different patient groups classified according to sex and estimated number of sickness points since the manifestation of the bronchial ithma. The difference between the individual values of MVV_{40} and the individual predicted values (predicted according to sex and age) are given. The significance of difference from the predicted value is stated with significance asteri ks Means of the individual ratios between observed and predicted values expressed in per cent are also given*

Maximum voluntary ventilation determined at a frequency chosen by the patient (MVV_f)

As may be seen in Table 15 the mean value for the maximum voluntary ventilation determined at a frequency chosen by the patient was 58.0 % of the predicted value for the men and 59.8 % for the women. The mean difference between the observed and the predicted value was −62.0 litres for the men and −42.0 litres for the women. This difference deviated significantly from zero both for the men and for the women (***).

The patients were classified into groups according to the estimated total number of sickness points since the onset of the asthma, and the quotient between the observed and the predicted value (expressed in per cent) was obtained for each patient. For those with fewer than 1000 points (group A) this mean value was 68.1 %. For those who had attained 1000 but not 2000 points (group B) a value of 54.1 % was obtained, and for those who had attained 2000 points or more (group C) 55.6 %.

The mean difference between group A and groups B and C combined, and also between group B and group C, were then calculated. The difference between A and B+C was found to be significant (*). No significant difference was found between B and C.

Patients according to sex and sickness points	Difference between observed MVV_f and predicted value (litres)										Difference from the predicted value (litres) mean	Percentage of predicted value mean
	No.	≥ +21	+20 +11	+10 −10	−11 −20	−21 −30	−31 −40	−41 −50	−51 −60	≤ −61		
Male	42	1	0	0	0	4	6	4	4	23	−62.0***	58.0
Female	74	0	1	5	5	10	11	13	17	12	−42.0***	59.8
Total	116	1	1	5	5	14	17	17	21	35	−49.7***	59.3
Group A												
Male	12	1	0	0	0	2	2	1	1	5	−18.5**	67.3
Female	26	0	1	3	3	4	5	4	5	1	−33.0***	68.5
Total	38	1	1	3	3	6	7	5	6	6	−27.9***	68.1
Group B												
Male	16	0	0	0	0	1	2	2	0	11	−60.1***	52.8
Female	25	0	0	0	1	4	2	7	5	6	−47.8***	55.0
Total	41	0	0	0	1	5	4	9	5	17	−52.2***	54.1
Group C												
Male	14	0	0	0	0	1	2	1	3	7	−63.2***	56.3
Female	23	0	0	3	1	2	4	2	7	5	−46.0***	55.2
Total	37	0	0	3	1	3	6	3	10	12	−53.3***	55.6

Table 15. Maximum voluntary ventilation determined at a frequency chosen by the patient (MVV_f) in different patient groups classified according to sex and estimated number of "sickness points" since the manifestation of the bronchial asthma. The difference between the individual value of MVV_f and the individual predicted values (prediction according to sex, age and height) are given. The significance of difference from the predicted value is stated with significance intervals. Means of the individual ratios between observed and predicted values expressed in per cent, are also given.

Total lung capacity (TLC)

Table 16 shows that the mean value for the total lung capacity was 94.4 % of the estimated normal value for the men and 93.6 % for the women. The mean difference between the observed and the predicted value was −0.4 litre for the men and −0.3 litre for the women. This difference deviated significantly from zero both for the men and for the women (*).

The patients were classified into groups according to the total number of sickness points since the onset of the asthma and the quotient between the observed and the predicted value (expressed in per cent) was obtained for each patient. For those with fewer than 1000 points (group A) this mean percentage value was 92.5. For those who had attained 1000 but not 2000 points (group B) this value was 94.2 %, and for those who had attained 2000 points or more (group C) 95.0 %.

The mean difference between group A and groups B and C combined, was then calculated. This difference, however, was not significant.

Functional residual capacity (FRC) in relation to total lung capacity (TLC)

Table 17 shows that the mean value for the functional residual capacity in relation to the total lung capacity was 111.3 % of the predicted value

Patients according to sex and sickness points	Difference between observed TLC and predicted value (litres)										Difference from the predicted value (litres) mean	Percentage of predicted value mean
	No	≥ +1.5	+1.4 +1.1	+1.0 +0.7	+0.6 +0.3	+0.2 −0.2	−0.3 −0.6	−0.7 −1.0	−1.1 −1.4	≤ −1.5		
M le	42	2	1	1	6	8	7	6	6	5	−0.4	94.4
Female	67	0	0	6	11	15	10	17	4	4	−0.3*	93.6
Total	109	2	1	7	17	23	17	23	10	9	−0.4***	93.9
Group A												
Male	12	0	0	0	3	1	2	2	3	1	−0.6	91.8
Female	24	0	0	1	4	5	5	6	2	1	−0.4	92.9
Total	36	0	0	1	7	6	7	8	5	2	−0.5	92.5
Group B												
Mal	16	1	0	0	2	3	3	3	1	3	−0.4	93.5
Femal	21	0	0	2	2	7	2	7	1	0	−0.3	94.8
Total	37	1	0	2	4	10	5	10	2	3	−0.3*	94.2
Group C												
Male	14	1	1	1	1	4	2	1	2	1	−0.2	97.7
Femal	22	0	0	3	5	3	3	4	1	3	−0.3	93.3
Total	36	1	1	4	6	7	5	5	3	4	−0.3	95.0

Table 10. *T tal lung capacity (TLC) in different patient groups, classified according t sex and estimated number of sickne points" since the manifestation of the bronchial asthma. The differences between the individual values of TLC and the individual predicted valu (predi t n according to sex age height and weight) are given. The significance of difference from the predicted value is stated with significance asterisks. Means of the individual ratios between observed and predicted values, expressed in per cent are also given.*

for the men and 121.6 % for the women. The mean difference between the observed and the predicted value was +0.1 per cent units for the men and +10.6 per cent units for the women. This difference deviated significantly from zero both for the men and for the women (*)

The patients were classified into groups according to the estimated total number of sickness points since the onset of the asthma, and the quotient between the observed and the predicted value (expressed in per cent) was obtained for each patient. For those with fewer than 1000 points (group A) this mean percentage value was 106.4 for the men and 114.8 for the women. For the men who had attained 1000 but not 2000 points (group B) a value of 112.1 % was obtained, and for the men who had attained 2000 or more (group C) 114.7 %. The corresponding values for the women were 124.4 % and 120.2 %

The mean difference between group A and groups B and C *combined*, and also between group B and group C, were then calculated. For the men the difference between A and B+C was not significant. For the women the corresponding difference was significant ( ) No significant difference was found between B and C, either for the men or the women.

Patients according to sex and sickness points	Difference between observed FRC/TLC and predicted value (per cent units)										Difference from the predicted value (per cent units) mean	Percentage of predicted value mean
	No	≥ +31	+30 +26	+25 +21	+20 +16	+15 +11	+10 + 6	+5 −5	− 6 −10	≤ −11		
Male	42	0	0	0	6	7	10	16	3	0	+ 8.1***	111.3
Female	67	0	2	5	10	17	12	18	2	0	+10.6***	121.6
Total	109	0	2	5	16	24	22	34	5	0	+ 8.9***	117.6
Group A												
Male	12	0	0	0	1	2	3	4	2	0	+ 2.4	106.4
Female	21	0	1	0	2	5	2	10	1	0	+ 7.3***	114.8
Total	33	0	1	0	3	7	5	14	3	0	+ 6.0***	112.0
Group B												
Male	16	0	0	0	2	4	3	6	1	0	+ 6.6**	112.1
Female	21	0	1	2	3	6	4	4	1	0	+12.0***	124.4
Total	37	0	1	2	5	10	7	10	2	0	+ 9.7***	119.1
Group C												
Male	14	0	0	0	3	1	4	6	0	0	+ 7.9**	114.7
Female	22	0	1	3	5	6	3	4	0	0	+13.0***	120.3
Total	36	0	1	3	8	7	7	10	0	0	+11.0***	121.7

Table 17. Functional residual capacity (FRC) in relation to total lung capacity (TLC) in different patient groups, classified according to sex and estimated number of sickness points since the manifestation of the bronchial asthma. The differences between the individual values of FRC/TLC and the individual predicted values (prediction according to sex, age and weight) are given. The significance of difference from the predicted value is stated with significance asterisks. Means of the individual ratios between observed and predicted values, expressed in per cent are also given.

Residual volume (RV) in relation to total lung capacity (TLC)

As may be seen in Table 18 the mean value for the residual volume in relation to the total lung capacity was 13/ 6 % of the predicted value for the men and 147 9 % for the women The mean difference between the observed and the predicted value was + 11 7 per cent units for the men and +14 6 per cent units for the women. This difference deviated significantly from zero both for the men and the women (***)

The patients were classified into groups according to the estimated total number of sickness points since the onset of the asthma and the quotient between the observed value and the predicted value (expressed in per cent) was calculated for each patient For those with fewer than 1000 points (group A) this mean percentage figure was 132 0 for the men and 13/ 5 for the women For those men who had attained 1000 but not 2000 points (group B) a value of 135.5 % was obtained, and for those who had attained 2000 points or more (group C) 144 7 % The corresponding values for the women were 153 4 % and 153 9 %.

The mean difference between group A and groups B and C combined, and also between group B and group C, were then calculated For the men the difference between A and B+C was not significant. For the women the corresponding difference was significant (*) No significant difference was found between B and C either for the men or the women.

Patient according to sex and sickness points	No	Difference between observed RV/TLC and predicted value (per cent units) ≥ +31	+30 +26	+25 +21	+20 +16	+15 +11	+10 + 6	+5 −5	− 6 −10	≤ −11	Difference from the predicted value (per cent units) mean	Percentage of predicted value mean
Male	42	1	3	3	2	10	14	9	0	0	+11 7***	137 6
Female	67	4	2	11	9	16	13	13	0	0	+14 6***	14 9
Total	109	5	5	14	11	26	27	21	0	0	+13.5 ***	143.9
Group A												
Male	12	0	1	1	1	3	1	5	0	0	+ 9 9**	132 0
Female	24	0	0	4	3	4	8	5	0	0	+11.5***	13 5
Total	36	0	1	5	4	7	9	10	0	0	+11.0**	135 7
Group B												
Male	16	0	1	2	0	3	6	4	0	0	+11.3**	135 5
Female	21	2	0	4	2	7	3	3	0	0	+16.1***	153 4
Total	37	2	1	6	2	10	9	7	0	0	+14.0***	145.7
Group C												
Male	14	1	1	0	1	4	7	0	0	0	+12.6***	144
Female	22	2	2	3	4	5	2	4	0	0	+16.6***	153.0
Total	36	3	3	3	5	9	9	4	0	0	+15.4***	150 3

Table 18. *Residual volume (RV) in relation to total lung capacity (TLC) in different patient groups classified according to sex and estimated number of sickness points since the manifestation of the bronchial asthma. The differences between the individual value of RV/TLC and the individual predicted values (prediction according to sex age height and weight) are given. The significance of difference from the predicted value is stated with significance asterisks. Means of the individual ratios between observed and predicted values expressed in per cent are also given.*

In summary it may be said that for each of the different indices of respiratory function the mean observed value for the patients in the asthma series differed significantly from the predicted value. The greatest percentage difference concerned the maximum voluntary ventilation, the forced expiratory volume per one second, and the ratio of residual volume to total lung capacity. The smallest difference concerned the total lung capacity and vital capacity.

When the patients were classified into groups according to the estimated degree of severity of the disease—based on the total number of sickness points — significant differences were found for several of the function variables between groups or combination of groups. The differences with the highest degree of significance were found in the tests indicating ventilation capacity. These differences were shown between the mean value for the group in which none of the patients had attained 1000 sickness points, and that for the group in which every patient had attained 1000 points or more. The only function variable for which a significant difference between the groups was not shown was the total lung capacity.

With regard to the ratios of the functional residual capacity and residual volume to total lung capacity the difference between the observed and the predicted values — calculated from the control material — was on an average somewhat greater for the women than for the men. As regards the other function variables no sex difference of any notable magnitude was found.

Wide ranges were found for each of the function variables both when the material was regarded as a whole and when it was divided into groups according to the estimated degree of severity of the disease.

Analysis of the relationship between some case history factors and different indices of respiratory function

An attempt was made to analyse the influence on the respiratory function of different case history factors of especial significance in bronchial asthma.

Table 19 shows the *mean values* of the respiratory function variables for the different patient groups distributed according to the case history factors. The latter factors comprised sex, age, estimated total number of sickness points, duration, age at onset, estimated total number of attack hours, estimated number of sickness points during the period 1957—1961 and the number of admissions to hospital on account of asthma. As seen in the table considerable differences were found. A more detailed significance analysis of the relationship between each lung function variable and each of the eight case history factors is given in Table 20.

Table 20 shows the degree of correlation of the case history factors with the lung function variables. Disregarding sex and age at onset, all of the factors are significantly correlated

Residual volume (RV) in relation to total lung capacity (TLC)

As may be seen in Table 18 the mean value for the residual volume in relation to the total lung capacity was 137 0 % of the predicted value for the men and 147 0 % for the women The mean difference between the ob served and the predicted value was + 11 7 per cent units for the men and +14 6 per cent units for the women. This difference deviated significantly from zero both for the men and the women (***)

The patients were classified into groups according to the estimated total number of sickness points since the onset of the asthma and the quotient between the observed value and the predicted value (expressed in per cent) was calculated for each patient For those with fewer than 1000 points (group A) this mean percentage figure was 132 0 for the men and 13, 5 for the women. For those men who had attained 1000 but not 2000 points (group B) a value of 135 5 % was ob tained, and for those who had attained 2000 points or more (group C) 144 7 % The corresponding values for the women were 153 4 % and 153 0 %

The mean difference between group A and groups B and C combined, and also between group B and group C, were then calculated For the men the difference between A and B+C was not significant. For the women the corresponding difference was signific ant (*) No significant difference was found between B and C either for the men or the women

Patients according to sex and sickness points	Difference between observed RV/TLC and predicted value (per cent units)										Differ ence from the predicted value (per cent nits) mean	Percent age of predicted alue mean
	No	≥ +31	+30 +26	+..3 +21	+20 +16	+15 +11	+10 + 6	+5 −5	− 6 −10	≤ − 11		
Male	42	1	3	3	2	10	14	9	0	0	+11 7***	13 6
Femal	67	4	2	11	9	16	13	12	0	0	+14 0***	14 9
Total	109	5	5	14	11	26	27	21	0	0	+13.5**	143 0
Group A												
Male	12	0	1	1	1	3	1	5	0	0	+ 9.9*	132.0
F male	24	0	0	4	3	4	8	5	0	0	+11.5**	13
Total	36	0	1	5	4	7	9	10	0	0	+11 0***	135 7
Gro p B												
Male	16	0	1	2	0	3	6	4	0	0	+11.3	13.. 5
F mal	31	2	0	4	2	7	3	3	0	0	+16.1***	133 4
Total	37	2	1	6	2	10	9	7	0	0	+14.0***	14..
Group C												
Male	14	1	1	0	1	4	7	0	0	0	+13.8***	144 7
Female	22	2	2	3	4	5	2	4	0	0	+16 6***	153 0
Total	36	3	3	3	5	9	9	4	0	0	+15.4***	150 3

T ble 18. *R sidual volume (RV) in relation to total lung capacity (TLC) in different patient groups classified according to sex and estimated number of ickn points since the manifestation of the bronchial asthma. The differe ces between the individual valu f RV/TLC and the individual predicted values (prediction according to ex age height and weight) ar given. The significance of difference from the predicted value i stated with ignificanc a terisks Means of th individual ratios between observed and predicted values expres ed in pe cent are also given.*

In summary it may be said that for each of the different indices of respiratory function the mean observed value for the patients in the asthma series differed significantly from the predicted value. The greatest percentage difference concerned the maximum voluntary ventilation, the forced expiratory volume per one second, and the ratio of residual volume to total lung capacity. The smallest difference concerned the total lung capacity and vital capacity.

When the patients were classified into groups according to the estimated degree of severity of the disease—based on the total number of sickness points — significant differences were found for several of the function variables between groups or combination of groups. The differences with the highest degree of significance were found in the tests indicating ventilation capacity. These differences were shown between the mean value for the group in which none of the patients had attained 1000 sickness points, and that for the group in which every patient had attained 1000 points or more. The only function variable for which a significant difference between the groups was not shown was the total lung capacity.

With regard to the ratios of the functional residual capacity and residual volume to total lung capacity the difference between the observed and the predicted values — calculated from the control material — was on an average somewhat greater for the women than for the men. As regards the other function variables no sex difference of any notable magnitude was found.

Wide ranges were found for each of the function variables both when the material was regarded as a whole and when it was divided into groups according to the estimated degree of severity of the disease.

Analysis of the relationship between some case history factors and different indices of respiratory function

An attempt was made to analyse the influence on the respiratory function of different case history factors of especial significance in bronchial asthma.

Table 19 shows the mean values of the respiratory function variables for the different patient groups distributed according to the case-history factors. The latter factors comprised sex, age, estimated total number of sickness points, duration, age at onset, estimated total number of attack hours, estimated number of sickness points during the period 1957—1961 and the number of admissions to hospital on account of asthma. As seen in the table considerable differences were found. A more detailed significance analysis of the relationship between each lung function variable and each of the eight case-history factors is given in Table 20.

Table 20 shows the degree of correlation of the case history factors with the lung function variables. Disregarding sex and age at onset, all of the factors are significantly correlated

Groups according to case history factors	Quantification	No	VC % of pred. y1	FEV1.0 % of pred. y2	FEV% % of pred y3	MVV40 % of pred. y4	MVVf % of pred. y5	No	TLC % of pred. y6	FRC/TLC % of pred. y7	RV/TL % pre y8
Sex											
Female	$X_1=0$	74	87 1	70.8	79.6	61.3	59.2	67	93.6	121.6	14
Male	$X_1=1$	42	85.0	70.0	79.8	60.3	58.0	42	94.4	111.3	13
Age											
60—68 yrs.	$X_2=1$	44	80.5	68.0	79 9	55.7	52.6	42	94.2	121.8	14
50—59 yrs	$X_2=2$	30	85.8	66.5	70.2	57.3	56.4	28	90.6	117.2	12
30—49 yrs.	$X_2=3$	42	92.7	76.0	81.9	68.9	68.1	39	96.1	113.8	11
Total sickness points											
<1000 p	$X_3=1$	38	89 6	78.6	86.3	68.4	68.1	36	92.5	112.0	13
1000—1999 p.	$X_3=2$	41	83.9	67.5	78.0	56.7	54.1	37	94.2	119.1	14
≧2000 p.	$X_3=3$	37	85.8	65.5	74.7	57.9	55.6	36	95.0	121 7	15
Duration											
<15 yrs.	$X_4=1$	32	88.9	77.9	87.0	63.7	62.9	30	91 7	111.8	13
15—24 yrs.	$X_4=2$	51	85.3	70 0	79.2	62.4	60.5	49	94.0	119.9	14
≧25 yrs.	$X_4=3$	33	85.4	64.0	73.4	55.9	53.5	30	96.1	120 1	14
Age at onset											
≦16 yrs.	$X_5=1$	22	92.9	76.6	81.6	69 6	67.6	21	96.3	112.2	13
17—39 yrs.	$X_5=2$	51	86.7	66.7	75 9	58.1	56.5	47	92.3	117 4	14
≧40 yrs.	$X_5=3$	43	82.5	71.8	83 3	59 7	58.3	41	93.6	120.7	14
Attack hours											
1—499 hrs.	$X_6=1$	37	87 7	74.4	82.9	66.0	64.2	35	94.1	115.2	14
500—2499 hrs.	$X_6=2$	32	85.8	72.0	81.8	59.9	60.3	29	93.9	117.0	14
≧2500 hrs.	$X_6=3$	44	85.8	66.1	75.2	57 1	54 1	42	93.8	119.9	14
Sickness points 1957—61											
< 50 p	$X_7=1$	70	87 4	73.6	82.6	63.4	67 3	65	94.4	115.3	11
50— 99 p	$X_7=2$	27	86 1	67.5	76.4	54 4	53.2	25	91.7	123.9	15
≧100 p	$X_7=3$	18	81 7	61 1	72.3	51.6	49.6	18	90.8	118.3	14
Admissions to hospital											
1—4 times	$X_8=1$	81	87.2	72.7	81.8	63.1	61.6	76	91 1	116.8	14
5—9 times	$X_8=2$	21	86.2	67 0	73.3	58 3	54.3	19	96.6	119.5	14
≧10 times	$X_8=3$	14	79 6	62.8	76.9	53.9	51 1	14	86.9	119 6	14
	Total	116	80.3	70.5	79 7	60.9	59.2	100[a]	93.9	117.6	14

Table 19 *Pe centage mean of the ratios between observed and predicted values for different indices the r pi atory function.*

Quantification as described on page 41 The individual value is formed as a ratio between the observed individual values and the predicted values multiplied by 100.

No = 116, except for attack hours No. = 113 and sickness points 1957—61 No. = 115
No = 109 except for attack hours No. = 106 and ickness points 1957—61 No. = 108

with at least two of these variables. The large number of significant correlations should be regarded in the light of the fact that the eight factors are themselves mutually correlated (see Table 7) and also that there is mutual correlation between the lung function variables (see Table 10).

Since the case-history factors show a high degree of mutual correlation, it *is difficult to interpret the significance* coefficients shown in Table 20. It may be considered that the relationships between certain factors and certain *lung function variables* are only apparent. In order to better evaluate the influence of the factors, a regression analysis was performed. The lung function variables were regarded as dependent variables and the eight case-history factors as independent. The relationship may be expressed as the following linear equation

$$y = a + b_1 X_1 + b_2 X_2 + \quad + b_8 X_8,$$

where y is lung function variable $\frac{\text{observed value}}{\text{predicted value}} \times 100$

a, b_1 b_2, b_3 b_8 the coefficients to be evaluated,

and X_1—X_8 the factors referred to in Table 19. Quantification was performed as shown by this table and also on page 41.

For the calculation of these coefficients there were 116 individual observations comprising data for X_1 X_2, X_3 X_8, and also the lung function variables (for TLC, FRC/TLC and RV/TLC, however, data were only obtained for 109 patients).

By means of this regression analysis the coefficients b_1 b_2 b_8 were estimated. The numerical results of the analysis are given in Table 21.

As a complement to Table 21 a similar regression analysis was performed

		VC y_1	FEV$_{1.0}$ y_2	FEV% y_3	MVV$_{40}$ y_4	MVV$_I$ y_5	TLC y_6	FRC/TLC y_7	RV/TLC y_8
Sex	X_1	−0.07	−0.01	0.06	−0.03	−0.01	0.03	−0.30**	−0.17
Age	X_2	[illegible]***	[illegible]*	0.04	0.32***	0.31***	0.06	−0.21	−0.06
Total sickness points	X_3	−0.11	−0.30**	−0.28**	−0.21	−0.26**	0.07	0.24	0.21
Duration	X_4	−0.09	−0.26**	−0.33***	−0.18	−0.23*	0.17	0.19*	0.15
Age at onset	X_5	−0.30**	−0.01	0.10	−0.14	−0.12	−0.10	0.16	0.00
Attack hours	X_6	−0.01	−0.16	−0.21	−0.20*	−0.20*	−0.01	0.10	−0.03
Sickness points 1957—61	X_7	−0.16	−0.29**	−0.28**	−0.35***	−0.30**	−0.09	0.14	0.12
Admissions to hospital	X_8	−0.15	−0.18	−0.17	−0.19*	−0.21	−0.10	0.07	0.00

Table 20. *Correlation coefficients — with significance asterisks — between case history factors and indices of respiratory function.*

Quantification of factors shown in Table 19 and described on page 41.

Groups according to case history factors	Quantification	No	VC %/o of pred. y_1	$FEV_{1.0}$ %/o of pred. y_2	FEV% /o of pred y_3	MVV_{40} %/o of pred. y_4	MVV_f %/o of pred. y_5	No.	TLC %/o of pred. y_6	FRC/TLC %/o of pred. y_7
Sex										
Female	$X_1=0$	74	87 1	70.8	79.6	61.3	59.8	67	93.6	121.6
Male	$X_1=1$	42	85.0	70.0	79.8	60.3	58.0	42	94.4	111.3
Age										
60—68 yrs.	$X_2=1$	44	80.5	68.0	79.9	55.7	52.6	42	94.2	121.6
50—50 yrs.	$X_2=2$	30	85.8	66.5	76.2	57.8	56.4	28	90.6	117.2
30—40 yrs.	$X_2=3$	42	92.7	76.0	81.9	68.9	68.1	39	96.1	113.6
Total sickness points										
<1000 p	$X_3=1$	38	89.6	78.6	86.3	68.4	68.1	36	92.5	112.0
1000—1999 p	$X_3=2$	41	83.9	67.5	78.0	56.7	54.1	37	94.2	119.1
≧2000 p	$X_3=3$	37	85.8	65.5	74.7	57.9	55.6	36	96.0	121 7
Duration										
<15 yrs.	$X_4=1$	32	88.9	77 9	87.0	63.7	62.9	30	91 7	111.6
15—24 yrs	$X_4=2$	51	86.3	70 0	79.2	62.4	60.5	49	94.0	119.9
≧25 yrs.	$X_4=3$	33	85 4	64.0	73.4	55.9	53.5	30	96.1	120 1
Age at onset										
≦16 yrs.	$X_5=1$	22	92.9	76.6	81.6	69 6	67 6	21	98.3	112.2
17—30 yrs.	$X_5=2$	51	86.7	66.7	75.0	58.1	56.5	47	92.3	117 4
≧40 yrs.	$X_5=3$	43	82.5	71.8	83.3	60 7	58.3	41	93.6	120.7
Attack hours										
1—490 hrs.	$X_6=1$	37	87 7	74.4	82.9	66.0	64.2	35	94.1	115 2
500—2490 hrs.	$X_6=2$	32	83.3	72.0	81.8	59 9	60.3	29	93.9	117.0
≧2500 hrs.	$X_6=3$	44	85.8	66.1	75.2	57 1	54.1	42	93.8	119.9
Sickness points 1957—61										
< 50 p.	$X_7=1$	70	87 4	73.6	82.5	65.4	67.3	65	94.4	115.3
50— 99 p.	$X_7=2$	27	86.1	67.5	76 4	54.4	53.2	25	94.7	123.9
≧100 p.	$X_7=3$	16	81.7	61 1	72.3	51.6	49.6	18	90.8	118.3
Admissions to hospital										
1—4 times	$X_8=1$	81	87.2	72.7	81.8	63.1	61 0	76	94 1	116.8
5—9 times	$X_8=2$	21	86.2	67.0	73.3	58.3	54.8	19	98.6	119.5
≧10 times	$X_8=3$	14	79 6	62.8	76.9	53.9	51 1	14	86.9	119.6
	Total	116	80.3	70.5	79 7	60.9	59 2	109[2]	93.9	117 6

Table 10 *P rcentage mean of the ratios between observed and predicted values for different indik the expiratory function.*

Quantification as described on page 41 The individual value is formed as a ratio between the observed, individual values and the predicted values multiplied by 100.

[1] No = 116, except for attack hours No = 113 and sickness points 1957—61: No = 113

[2] N = 109 except for attack hours: No = 106 and sickness points 1957—61 No. = 108

lysis table (Table 22). In the first analysis (Table 21) the age at onset and duration are also shown as factors of some influence. In consideration of the fact that age, age at onset and duration show a high degree of mutual correlation (multicollinearity) however greater regard should be paid to the results given in Table 22 with respect to the coefficients for age and duration.

With regard to the variables FEV $_{x}$ and FEV $_{\%}$ the duration and sickness points during the period 1957—61 are shown to be of some influence, which was only significant, however for FEV x (see Table 21). The total sickness points appeared to be of little importance, at least as shown by the analysis in which the points for 1957—61 were included. When the points for this period were not included as a separate variable (see the second regression analysis, Table 22) the total sickness points then had higher values of coefficiency. This may indicate that the correlation of the total sickness points to lung function reduction depended essentially on the intensity of the disease during the years almost immediately preceding the investigation. (The intensity of the disease = total number of sickness points during the period referred to divided by this period expressed in years.)

To elucidate this question further FEV was correlated to the intensity of the disease from its onset up to the end of 1956. This correlation was –0.10, i.e. weak, which suggested that the intensity of the disease during the period before 1957 was of only minor influence at least to this lung function variable. A corresponding calculation between FEV x and the intensity in 1957—61 showed a correlation of up to –0.33 which indicates that the intensity of the disease during the more recent years was significantly (*) correlated to this lung function variable. The partial correlation between the disease intensity before 1957 and the lung function as measured by FEV x at the present investigation—eliminating the influence of the disease intensity of 1957–61—was weak, i.e. 0.01.

An analogous calculation with regard to FEV $_{\%}$ showed that the partial correlation was of the same order of size.

With regard to the variables MVV $_{\%}$ and MVV $_{l}$ it was found in the first regression analysis that the sickness points during the period 1957—61 were of significant influence. Duration, age and number of attack hours also, however had some influence. The total sickness points, on the other hand, were found in the first analysis to be of no appreciable importance (Table 21).

In the second limited analysis (Table 22) the age factor was found to be significant, and some influence could also be ascribed to the total number of sickness points. The duration, on the other hand, was of less importance. In calculating the predicted values consideration was taken of the fact that in the control material also the test performance would have decreased with increasing age even in normal persons these two lung function variables in particular are strongly dependent on age.

The degree of correlation between

but only the following four factors were studied sex age, sickness points and duration. The results are given in Table 22 The motivation for this limited version was to obtain firstly a more general analysis comprising only the more "rough factors, and secondly an analysis in which the age at onset was not included — since this age was practically identical with the actual age of the patient minus duration similarly in years.

The *analysis shows that as regards* the first lung function variable VC, the factors found typically in the case histories of asthma patients were of no appreciable influence. Only the age factor appears to have any noteworthy effect — but not significant — and this is shown by the second regression ana

Independent variable	Variable notation	Notation of regr. coeff	Dependent variables							
			VC	$FEV_{1.0}$	FEV %	MVV_{40}	MVV_f	TLC	FRC/TLC	RV/TLC
Sex	X_1	b_1	—1.9	0.1	0 4	—0.3	—1.0	1 1	—9.9	—9 7
Age	X	b_2	3.3	1 7	—1 7	4 0	5 0	1.5	0.3	3.9
Total sickness points	X_3	b_3	0 1	—1.2	0.5	0.7	0 4	1 1	4 2	12.5**
Duration	X_4	b_4	—3.2	—5.8	—7.8	—4 1	—5 7	3.9	5.5	5.3
Age at onset	X_5	b_5	—1.0	—3.0	—3.7	—3.8	—3.8	1.2	6	0
Attack hours	X_6	b_6	—0 6	—1 1	—2.0	—3.3	—3.2	—0 6	—0 1	—0.8
Sickness points 105 —01	X_7	b_7	—1.5	—4.5	—4.5	—6.7	—6 7*	—1.6	1 2	2.3
Admission to hospital	X_8	b_8	—1 1	—0.8	—0 4	0 4	—0.6	—1.9	—0 2	—1.5

Table 21 *Coefficients calculated in regression analyses comprising eight factors and — dependent variable — lung function indices*
Quantification as described on page 41

Independent variable	Variable notation	Notation of regr coeff	Dependent variables							
			VC	$FEV_{1.0}$	FEV %	MVV_{40}	MVV_f	TLC	FRC/TLC	RV/TLC
Sex	X_1	b_1	—1.5	0.2	0 6	—0 1	0.8	—0 8	—10 0	—10.8
Age	X_2	b_2	5.3	3.5	0.3	6.0	7 1 **	1.0	—3 8*	—2 1
Total sickness points	X_3	b_3	0	—3.5	— 2 4	—3.8	—3 6	—0 5	3.7	0.7
Duration	X_4	b_4	—0 7	—3.8	—5.3	—1.2	—2.9	3 5	1 0	1 6

Table 22 *Coefficients calculated in regression analyses comprising four of the eight factors included in Table 21 and — as dependent variable — different lung function indices*
Quantification as described on page 41

ards the ratio between the residual volume and total lung capacity the total number of sickness points for the whole period after the onset of the asthma, and sex, appeared to be of noteworthy influence. The relationship between the total number of sickness points and the RV/TLC ratio did not thus appear to depend essentially on the intensity of the disease during the years almost immediately preceding the investigation, as seemed to be the case with the ventilation capacity

---

Patients with a chronic disease in addition to the bronchial asthma

Of the 126 patients in the asthma series, 12 (6 men nd 6 women) were diagnosed as having chronic disease in addition to the bronchial asthma. Since it wa considered that this disease might influence the results of the analyses, these patients have been reported separately A they were relatively mall in number and no significant differences were shown between the different sex and age groups, the results are presented independently of these tw f ctors.

In Table 23 these 12 patients are distributed into groups ccording to the estimated total number of sickness points (see page 25) for bronchial asthma since the onset of this disease. The majority of the patient are as igned to group C, i.e. they had tained 2000 points or more. The mean number of sickness points for these 12 patient in elation t the rest f the asthma series is somewhat high (cf Table 2)

Table 24 show the mean values nd ranges for the entilation capacity nd lung olumes, a percentage of the predicted values, for these 12 patients. If this table is compared with Table 15, it is seen that the mean lue of the riables for the different tests of ntilation capacity is lower for this group of patient than for the rest of the asthma series. With regard to the lung volumes nd the mutual relationships between them, this group differs somewhat les on an verage from the rest of the material.

Group according to sickness points	No.	Sickness points mean	Sickness points range
A (<1000 p.)	2	464	432— 495
B (1000—1999 p.)	3	1406	1318—1653
C (≥2000 p.)	7	2863	2034—3858
Total	12	2138	432—3858

Table 23. *Distribution (a) groups according t estimated total number f sickness points for 12 pat ents who besides bronchial asthma had another chronic disease*

	FEV1.0 % of pred	FEV% % of pred.	MVV40 % of pred.	MVVf % of pred.
mean	43.0	87.4	38.0	33.1
range	19.1—70.0	41.7—120.8	23.3—60.4	20.1—50.1

	VC % of pred.	TLC % of pred.	FRC/TLC % of pred.	RV/TLC % of pred.
mean	60.0	81.9	131.2	167.7
range	37.8—81.8	48.5—116.4	113.2—145.1	133.8—237.7

T ble 24. *Mean val es and ranges of ventilation capacity and lung volume for 12 patients with chron disease besides bronchial asthma, as percentage of the predicted value (prediction according to sex, age, height and weight)*

the lung function variable MVV_f and the estimated number of attack hours during the five years 1957–61 was studied. This was not found to be especially high: the correlation was –0.20 (*) but that between the number of attack hours before 1957 and the variable MVV_f was only slightly less, i. e. –0.16.

With regard to the variable TLC, the only factor of any influence — not, however significant — was the duration, and this is shown by both analyses (Tables 21 and 22).

With regard to the variables FRC/TLC and RV/TLC, both regression analyses showed that sex and the total sickness points had considerable influence. According to the first analysis the age at onset and duration were also of importance. Special attention should be paid to the fact that for both FRC/TLC and RV/TLC the total sickness points were shown to be of some influence (significant for RV/TLC) even in the analysis where the points for the period 1957—61 were regarded as an independent variable. This appears to indicate that the correlation of the sickness points with the variables FRC/TLC and RV/TLC — in contrast to that with $FEV_{1.0}$, FEV %, MVV_{40} and MVV_f — depended essentially on the over all severity of the disease i. e. the total number of sickness points since the beginning of the bronchial asthma. In the case of the latter variables, on the other hand, the correlation of the total sickness points to the reduced lung function appeared to depend essentially on the intensity of the asthma during the years 1957—61.

To summarize these regression analyses indicate a considerable relationship between the reduction in ventilation capacity and the estimated severity of the disease during the years almost immediately preceding the investigation. If the sickness points for these later years were not regarded as an independent factor in the analysis, the total sickness points, i. e. the estimated over all degree of severity of the disease since its onset emerged as a factor of noteworthy influence. (See Table 20 which shows the correlation between pairs of X and y variables.) The correlation of the total sickness points with the lung function reduction thus appears to depend essentially on the degree of severity of the asthma during the years recently preceding the investigation.

With regard to the ventilation capacity — as estimated from the maximum voluntary ventilation, i. e. the function variables MVV_{40} and MVV_f —age appeared to be an essential factor, this capacity decreasing with increasing age. Age which influenced the performance even of persons in the control material, appeared to have even greater weight as regards the asthma series.

This was also valid to a certain extent for the relationship between age and vital capacity. The value for the total lung capacity appeared to increase with the length of duration of the disease. These relationships were not significant. With regard to the ratio between the functional residual capacity and total lung capacity noteworthy influence could be ascribed to sex, age and possibly total number of sickness points and duration. As reg

made, group C differed significantly ( ) from group A.

When the material was divided into three groups according to the *duration* of the asthma (Table 26) (group 1 duration of <15 years, group 2 15—24 years, group 3 ≥25 years) the observed mean value for the men in both group 1 and group 3 differed significantly (* and ** respectively) from the predicted value.

With regard to the women, all groups differed significantly from the predicted value (group 1 group 2 and group 3 *)

Neither for the men nor for the women, nor when the sexes were combined was there a significant mutual difference between the three groups or between combinations of the groups.

When the material was divided into three groups (Table 27) according to the *age at onset* (group 1 onset before or at the age of 16 years, group 2 onset after the age of 16 years but before the age of 40 years, group 3 onset at or after the age of 40 years) the observed mean value for the men in groups 2 and 3 differed significantly ( ) from the predicted value. The difference with regard to the men in group 1 was not significant. No significant difference was found between the different groups.

Groups according to estimated total No. of sickness points	Sex	Work load, percentage of predicted value at: heart rate 130 beats/min — No.	mean	range	SD	SE	% of patients at steady state	maximum performed work — No.	mean	range	SD	SE	% of patients at steady state
<1000 p	M	11	93.0	38.6—130.8	31.2	9.4	82	11	93.6	45.0—133.5	25.5	7.7	55
1000—1999 p	M	16	74.1	36.9—121.5	22.6	5.7	75	16	80.1	41.0—118.1	20.2	5.1	81
≥2000 p	M	14	78.9	36.6—141.6	24.3	6.6	61	14	73.9	35.8—118.1	27.4	7.8	43
<1000 p	F	25	82.0	38.5—163.[illegible]	30.9	6.2	80	25	79.1	40.0—130.3	21.9	4.4	82
1000—1999 p	F	24	78.5	30.2—120.6	30.5	6.2	67	23	83.2	30.8—141.1	25.6	5.3	68
≥2000 p	F	21	82.5	42.6—121.7	22.4	4.9	62	23	80.7	9.0—131.9	25.5	5.9	57

Table 25. W_{130} and W_{max} as percentage of predicted value, for the material classified into groups according to sex and estimated total number of sickness points since the manifestation of the asthma.

Groups according to duration of the bronchial asthma	Sex	Work load, percentage of predicted value at: heart rate 130 beats/min — No.	mean	range	SD	SE	% of patients at steady state	maximum performed work — No.	mean	range	SD	SE	% of patients at steady state
<15 yrs	M	11	74.7	38.5—121.5	22.0	8	91	11	90.8	53.0—123.5	20.1	6.1	82
15—24 yrs	M	17	86.3	38.9—141.6	29.2	7.1	85	17	80.5	41.0—123.3	28.	7.0	71
≥25 yrs	M	13	[illegible]6.3	35.6—106.5	19.2	5.3	00	13	74.3	35.8—109.6	22.0	6.1	31
<15 yrs	F	20	84.7	30.5—163.6	29.4	6.6	45	20	78.0	40.0—130.3	23.2	5.2	65
15—24 yrs	F	33	79.1	30.2—131.4	28.8	5.1	60	31	73.8	9.0—161.1	26.8	4.0	59
≥25 yrs	F	18	79.5	43.8—129.6	23.1	5.5	61	19	83.7	40.0—135.1	27.4	6.3	63

Table 26. W_{130} and W_{max} as percentage of predicted value for the material classified into groups according to sex and duration of the bronchial asthma.

DETERMINATION OF PHYSICAL WORK CAPACITY WITH SUBMAXIMAL WORK TEST

The physical work capacity was determined for all the 128 patients in the asthma series. The 12 patients who in addition to bronchial asthma had another chronic disease of significance are reported separately (see page 71). Tables 31 and 32 (pages 72–75) show all the variables recorded or calculated in connection with the work test both for the entire material distributed according to sex and age, and also for the different groups according to the estimated total number of sickness points. Tables 25—30 show the work load given in per cent of predicted kpm/min calculated from the observed data by interpolation or extrapolation (= rate of work) at heart rate 130 (W_{130}) and also the maximum work performed at the work test (W_{max}) for the asthma series grouped according to different case history data e g duration, age at onset etc. Figs 2—4 show the relationship between the calculated physical work capacity at heart rate 130 with both the total hemoglobin content and the cardiac volume in the prone position on classification of the patients according to the estimated total number of sickness points. For the method used in the determination of circulatory functions reference may be made to page 27.

The results from both W_{130} and W_{max} will be described separately below. In both cases comparison is made with the control material, and the influence of different case history data is discussed. Comparison is also made between the asthma series and the control material with regard to the other variables recorded or calculated in connection with the work test.

Work load at heart rate 130 (W_{130})

The difference between the observed mean value expressed as a percentage of the predicted value, and this latter value (100 %) was significant for both men and women (***). No significant difference was found between the three age groups.

On classification of the material into three groups (Tables 25, 31—32) according to the estimated total number of sickness points (group A <1000 points, group B 1000—1999 points, group C ≧2000 points, see page 35) the mean value for the men in group A was somewhat lower than, but did not differ significantly from the predicted value, while the values for the men in groups B and C, on the other hand, were significantly lower than predicted (***). Groups B and C had a lower mean value than group A but did not differ significantly either from group A or from each other; neither did the combined groups B and C differ significantly from group A.

With regard to the women each of the groups differed significantly from the predicted value (group A **, group B and group C ***). The groups did not differ significantly from one another and neither did the combined groups B and C differ significantly from group A.

When no sex classification was

into three groups (Table 29) according to the mean *number of sickness points per year during the 5-year period 1957—61* (group 1 <60 points, group 2 60—99 points, group 3 ≥100 points) the mean value for the men in all groups differed significantly from the predicted value (* and *** for groups 1 2 and 3 respectively)

With regard to the women, groups 1 and 3 differed significantly from the predicted value (* and ** respectively)

Neither for the men nor for the women, nor when the sexes were combined was there a significant mutual difference between the three groups or between combinations of the groups.

When the material was classified into two groups (Table 30) according to the *number of admissions to hospital* on account of bronchial asthma (group 1 1—4 admissions, group 2 5 or more admission) the mean value of both the men in the two groups and the women in the two groups differed *significantly (*) from the predicted* value.

There was no significant mutual difference between the groups for either sex. No significant difference was found between the two groups when the sex factor was disregarded

Groups according to estimated No of sickness point for 1957—61	Sex	Work load, percentage of predicted value at: heart rate 130 beats/min						maximum performed work					
		No.	mean	range	SD	SE	% of patients at steady state	No.	mean	range	SD	SE	% of patients at steady state
< 60 p.	M	22	84.8	38.8—141.6	27.3	5.8	83	22	87.0	41.0—123.3	23.8	5.1	55
60— 99 p.	M	10	80.6	36.9—118.5	25.1	7.9	60	10	[illegible]	35.8—107.0	21.5	6.8	70
≥100 p.	M	8	65.2	38.5— 96.9	20.2	7.2	75	8	68.9	38.0—109.5	24.5	8.7	75
< 60 p.	F	45	73.8	26.8—131.4	28.1	3.9	62	46	78.7	40.0—136.2	23.9	3.5	52
60— 99 p.	F	14	87.4	29.2—163.5	36.5	9.1	60	17	82.2	36.8—151.1	26.2	6.2	71
≥100 p	F	9	70.8	43.8—102.2	22.7	7.6	67	10	73.4	9.0—131.9	23.5	11.2	70

Table 29. *W_{130} and W_{max} as percentage of predicted value for the material classified into groups according to sex and estimated average number of sickness points per year for the period 1957—61*

Groups according to No. of times admitted to hospital for bronchial asthma	Sex	Work load, percentage of predicted value at: heart rate 130 beats/min						maximum performed work					
		No.	mean	range	SD	SE	% of patients at steady state	No.	mean	range	SD	SE	% of patients at steady state
1—4 times	M	27	83.9	38.5—141.5	26.0	5.0	82	27	84.5	41.0—123.5	23.0	4.4	78
≥5 times	M	14	74.9	36.0—121.5	27.5	7.3	57	14	75.1	33.8—118.1	27.8	7.7	43
1—4 times	F	49	78.2	27.2—163.5	31.1	4.4	67	52	77.5	9.0—151.1	27.0	3.7	60
≥5 times	F	21	74.8	36.8—116.8	22.2	4.9	52	21	77.7	40.0—102.8	24.1	5.3	67

Table 30. *W_{130} and W_{max} as percentage of predicted value for the material classified into groups according to sex and number of times admitted to hospital for bronchial asthma*

With regard to the women the observed mean value for all three groups differed significantly from the predicted value (group 1 *** group 2 ** group 3 *) Groups 1 and 2 combined differed significantly (*) from group 3

When no sex classification was made group 3 differed significantly (*) from group 1 Otherwise the difference between the groups was not significant

When the material was classified into three groups (Table 28) according to the estimated number of *attack hours* (group 1 1—499 hours group 2 500—2499 hours group 3 ≧2500 hours) the observed mean value for the men in *group 1 did not differ* significantly from the predicted value while the mean value for those in groups 2 and 3 differed significantly (***)

With regard to the women, each of the groups differed significantly from the predicted value (***)

There was no significant mutual difference between the three groups or between combinations of the groups either for the men or for the women. There was still no significant mutual difference between the groups when the sex factor was disregarded.

When the material was classified

Groups according to age at manifestation of bronchial asthma	Sex	Work load, percentage of predicted value at heart rate 130 beats/min: No	mean	range	SD	SE	% of patients at steady state	Work load, percentage of predicted value at maximum performed work: No	mean	range	SD	SE	% of patients at steady state
≦10 yrs.	M	11	83.8	60.0—141.5	25.9	7.8	82	11	8.1	33.8—123.3	26.1	.9	36
17—39 yrs.	M	16	8 0	38.5—121.5	.5.4	6.4	63	16	82.3	38.6—118 1	21.6	5.4	63
≧40 yrs.	M	14	7 1	36.9—121.5	28.7	7 7	79	14	82.7	41.0—125.5	28	7	79
≦10 yrs.	F	10	71.3	38.9—111 4	24 4	7 0	80	10	74.3	45.5—165 9	17.6	3.6	60
17—39 yrs.	F	53	72.3	20.2—176.6	27 1	4 7	68	25	73.6	9.0—136.1	23.4	4.5	54
≧40 yrs.	F	27	83.5	36.5—16..5	30 7	5.9	03	28	83.7	36.8—151.1	28	5.4	61

Table 27 W_{130} and W_{max} a pe entage of predicted valu for the material classified into gr ups according to sex and age at manifestati n f bronchial a thma

Groups according to estimated total N of attack hours	Sex	Work load, percentage of predicted value at heart rate 130 beats/min: No	mean	range	SD	SE	% of patients at steady state	Work load, percentage of predicted value at maximum performed work: No	mean	range	SD	SE	% of patients at steady state
1—499 hrs.	M	12	90.8	38.5—130.8	27.3	7 9	83	12	92.9	38 7—1.3.5	22.4	6.8	67
500—2499 hrs.	M	10	73.5	44.0—118.5	22.5	7 1	90	10	77.9	41.0—109 5	20.6	6.5	70
≧2500 hrs.	M	17	8.3	36.0—141.5	26 9	6.5	59	17	72.6	3.8—118.1	26.0	6.3	47
1—499 hrs.	F	23	78.3	36.3—131 4	30.0	6.2	03	25	82 0	9.0—151 1	33.8	6 8	46
500—2499 hrs.	F	19	77.5	36.5—121 1	24.4	5.6	53	20	70 8	43.3—101.0	18.1	4.0	45
≧2500 hrs.	F	37	78.0	20.2—121 7	26.0	5.0	07	27	3.6	40 0—131.9	23.1	4.6	61

Table 28. W_{130} and W_{max} as percentage f predicted valu f r the material classified int group according to sex and timated t tal numbe f attack h urs since the manifestation f th bronchial asthma

into three groups (Table 29) according to the mean *number of sickness points per year during the 5-year period 1957—61* (group 1 <50 points, group 2 50—99 points, group 3 ≧100 points) the mean value for the men in all groups differed significantly from the predicted value (* and for groups 1, 2 and 3 respectively).

With regard to the women, groups 1 and 3 differed significantly from the predicted value (*** and * respectively).

Neither for the men nor for the women, nor when the sexes were combined was there a significant mutual difference between the three groups or between combinations of the groups.

When the material was classified into two groups (Table 30) according to the *number of admissions to hospital* on account of bronchial asthma (group 1 1—4 admissions, group 2 5 or more admissions) the mean value of both the men in the two groups and the women in the two groups differed significantly ( ) from the predicted value.

There was no significant mutual difference between the groups for either sex. No significant difference was found between the two groups when the sex factor was disregarded.

Groups according to estimated No. of sickness points for 1957—61	Sex	Work load, percentage of predicted value at: heart rate 130 beats/min						maximum performed work					
		No.	mean	range	SD	SE	% of patients at steady state	No.	mean	range	SD	SE	% of patients at steady state
<50 p.	M	22	81.8	35.5—141.5	27.3	5.8	82	22	87.0	41.0—125.3	23.8	5.1	[illegible]
50—99 p.	M	10	80.6	38.9—118.6	25.1	7.9	60	10	4.5	35.8—102.0	21.3	6.8	70
≧100 p.	M	8	65.2	35.—96.9	20.2	7.2	75	8	68.9	35.6—100.5	24.5	8.7	75
<50 p.	F	45	73.8	36.8—131.4	20.1	3.0	62	46	78.7	40.0—136.3	23.9	3.5	52
50—99 p.	F	16	87.4	39.3—143.8	36.3	9.1	63	17	82.2	36.8—181.1	29.2	6.2	71
≧100 p.	F	9	76.8	43.8—103.2	22.7	7.6	67	10	73.4	9.0—131.9	35.5	11.2	78

Table 29. W_{130} and W_{max} as percentage of predicted value for the material classified into groups according to sex and estimated average number of sickness points per year for the period 1957—61

Groups according to No. of times admitted to hospital for bronchial asthma	Sex	Work load, percentage of predicted value at: heart rate 130 beats/min						maximum performed work					
		No.	mean	range	SD	SE	% of patients at steady state	No.	mean	range	SD	SE	% of patients at steady state
1—4 times	M	37	83.9	30.5—141.5	26.0	5.0	82	37	81.3	41.0—123.5	23.0	4.4	70
≧5 times	M	14	71.9	36.9—121.5	27.5	7.3	57	14	73.1	33.8—118.1	27.8	7.7	43
1—4 times	F	49	78.3	20.3—163	31.1	4.4	67	53	77.5	9.0—151.1	27.0	3.7	60
≧5 times	F	21	71.9	36.6—116.8	23.3	4.9	62	21	77.7	40.0—103.0	24.1	5.3	67

Table 30. W_{130} and W_{max}, as percentage of predicted value for the material classified into groups according to sex and number of times admitted to hospital for bronchial asthma.

With regard to the women the observed mean value for all three groups differed significantly from the predicted value (group 1 *** group 2 ** group 3 *) Groups 1 and 2 combined differed significantly (*) from group 3

When no sex classification was made group 3 differed significantly (*) from group 1 Otherwise the difference between the groups was not significant

When the material was classified into three groups (Table 28) according to the estimated number of *attack hours* (group 1 1—499 hours group 2 *500—2499* hours, group *3* ≥2500 hours) the observed mean value for *the men in group 1 did not differ* significantly from the predicted value, while the *mean value for those in* groups 2 and 3 differed significantly (***)

With regard to the women each of the groups differed significantly from the predicted value (***)

There was no significant mutual difference between the three groups or between combinations of the groups either for the men or for the women. There was still no significant mutual difference between the groups when the sex factor was disregarded

When the material was classified

Groups according to age at manifestation of bronchial asthma	Sex	Work load percentage of predicted value at heart rate 130 beats/min						Work load percentage of predicted value at maximum performed work					
		No	mean	range	SD	SE	% of patients t steady state	No	mean	range	SD	SE	% of patients at steady state
≤16 yrs.	M	11	88.8	60 0—141.5	25.0	7.8	82	11	78.1	33.8—125.3	26.1	.9	36
17—39 yrs.	M	16	8.0	38.5—121.5	23.4	6.4	63	16	82.3	36.6—118.1	21.6	5.4	83
≥40 yrs.	M	14	77 1	36.0—121.5	28.7	7 7	79	14	82.7	41.0—125 5	28.		79
≤16 yrs.	F	10	71.3	38.9—114 4	21 4	7 9	80	10	74.3	45.5—101 0	1 .8	5.6	80
17—39 yrs.	F	33	72.3	20.2—126.5	27 1	4.7	58	25	73.6	9 0—186.1	23.4	4 3	51
≥40 yrs.	F		85.5	36.5—163.5	30 7	5.9	63	28	83.7	36 8—161 1	28	5.4	64

Table 27 W_{130} and W_{max} a percentage of predicted value for the material classified int groups according to ex and age at manifest tion of bronchial asthma

Groups according to estimated total No of ttack hours	Sex	Work load, percentage of predicted val e at heart rate 130 beats/min						Work load, percentage of predicted val e at maximum performed w rk					
		No	mean	range	SD	SE	% of patients t steady state	No	mean	range	SD	SE	% of patients at steady state
1—499 hrs.	M	12	90.8	38.5—130.5	27.3	7 9	83	12	92.0	58.7—1[illegible]3 5	23 4	6 8	67
500—2499 hrs.	M	10	73.5	44.6—118.5	22.5	7 1	90	10	77.9	41 0—100.[illegible]	20 6	6.5	70
≥2500 hrs.	M	17	70.3	36.9—141.5	26.9	6.5	59	17	72.6	35 8—118.1	26 0	6 3	47
1—499 hrs	F	23	78.3	36.3—131.4	30.0	6.2	65	23	82.0	9.0—151 1	33 8	6 8	56
500—2499 hrs.	F	19	77.5	36.3—124 1	24.4	5.6	53	20	76 8	43 3—101.0	18 1	4 0	53
≥2500 hrs.	F	27	73.0	20.2—121 7	26 0	5.0	67	27	73 6	40.0—131.9	23 1	4.5	63

Table 28. W_{130} and W_{max} as percentage f predicted valu for the material classified int group according to ex and e timated total number of attack hours ince the manifestati n f the bron hial a thma.

the men in groups 2 and 3 showed a significant difference ( ) Group 3 differed significantly ( ) from group 1 When groups 2 and 3 were combined, the difference between their weighted mean value and the value for group 1 was significant ( )

With regard to the women, each of the groups differed significantly from the predicted value (group 1 group 2 and group 3 *) There was no significant mutual difference between the groups, and neither did group 1 differ significantly from the combined groups 2 and 3

When no sex classification was made, the difference between group 3 and group 1 was significant ( ) The difference between group 1 and the combined groups 2 and 3 was also ignificant ( )

When the material was classified into three groups (Table 29) according to the mean number of *sickness points per year during the 5-year period 1957—61* the mean value for the men in all three groups differed significantly from th predicted value (group 1 group group 3 **) No sign ificant mutual difference between the groups was found, but a significant difference ( ) was seen between group 1 and the combined groups 2 and 3.

With regard to the women, all three groups differed significantly from the predicted value (group 1 group 2 group 3) There was no sign ificant difference between the groups.

When no sex classification was made no significant difference between the groups was found.

On classification of the material into two groups (Table 30) according to the number of admissions to *hospital* on account of bronchial asthma, the mean value for both the men in the two groups (*) and the women in the two groups (***) differed significantly from the predicted value Neither for the men nor for the women was there any significant difference between the two groups.

No significant difference was found between the two groups when the sex factor was disregarded.

Relationship between physical work capacity and lung function

The correlation between work capacity (W_{170} and W_{max}) and different lung function variables was studied For the variables of both work capacity and lung functions, each patient is represented by the quotient between the observed and predicted value

The study showed that W_{170} was highly significantly correlated with VC (correlation coefficient, r= 0.48) Further significant relationships were found with $FEV_{1.0}$, $FEV_{\%}$, MVV_{40}, MVV_{f} (r=0.30—0.33) and with FRC/TLC (r=0.20) No sign ificant relationship was found between W_{170} and RV/TLC.

A similar study was made with reg ard to W_{max} This showed that W_{max} was significantly correlated with VC, $FEV_{1.0}$, MVV_{40} and MVV_{f} (r=0.28—0.31) The correlation between W_{max} and $FEV_{\%}$ was also significant (=0.22) No significant relationship was found between W_{max} and FRC/TLC or RV/TLC.

Maximum performed work load (W_{max})

The difference between the observed mean value expressed as a percentage of the predicted value and this latter value (100 %) was significant (***) for both sexes. For the women there was a significant difference (*) between the youngest and the oldest group the latter group having on an average a higher value. For both sexes combined the difference between the oldest and middle age groups was significant (*) the former having on an average a higher value. No other significant difference was shown between the three age groups.

When the material was classified into three groups (Tables 25 31–32) according to the estimated *total number of sickness points* the mean value for the men in group A did not differ significantly from the predicted value while the mean value for the men in groups B and C showed a significant difference (***) There was no significant mutual difference between the groups and neither was there any significant difference between group A and groups B and C combined.

With regard to the women each of the groups differed significantly from the predicted value (group A and group C *** group B **) There was no significant mutual difference between the groups, and neither did group C differ significantly from groups A and B combined

On classification of the material regardless of sex, a significant difference (*) was found between groups C and A The difference between groups B and A on the other hand, was not significant. No significant difference was shown between group C and groups A and B combined.

When the material was classified into three groups (Table 26) according to the *duration* of the asthma the observed mean value for the men in groups 2 and 3 differed significantly from the predicted value (** and *** respectively)

With regard to the women, the mean value for all groups differed significantly from the predicted value (group 1 and group 2 *** group 3 *)

Neither for the men nor for the women, nor when the sexes were combined was there a significant mutual difference between the three groups or between combinations of the groups.

When the material was divided into three groups (Table 27) according to the *age at onset* the observed mean value for the men in groups 1 2 and 3 differed significantly (** ** and * respectively) from the predicted value.

For the women also the observed mean value for all three groups differed significantly from the predicted value (group 1 *** group 2 *** group 3 **)

There was no significant mutual difference between the three groups or between combinations of the groups for either sex. There was still no significant difference between the groups when the sex factor was disregarded.

When the material was divided into three groups (Table 28) according to the estimated number of *attack hours* the observed mean value for the men in group 1 did not differ significantly from the predicted value, while that for

force maximal exertion (and neither was this the intention) but that a relatively high degree of exertion was attained.

On classification of the asthma series into groups according to sex, age and estimated total sickness points, no significant difference was found for the above variables between these groups (except for the highest heart rate attained, which was dependent on age). Neither was any significant difference found between the control material and the asthma series on comparison between the sex and age groups.

For W_{130} a mean value of 721 kpm/min (S D=163 kpm/min, S E=31 kpm/min) was obtained for the men in the asthma series, and of 437 kpm/min (S D=113 kpm/min, S E=16 kpm/min) for the women. For the men in the control material a mean figure of 851 kpm/min (S D=161 kpm/min, S E=28 kpm/min) was obtained, and for the women 517 kpm/min (S D=142 kpm/min, S E=21 kpm/min).

For W_{170} a mean figure of 876 kpm/min (S D=180 kpm/min, S E=37 kpm/min) was obtained for the men in the asthma series, and of [illegible] kpm/min (S D=122 kpm/min, S E=20 kpm/min) for the women. For the men in the control material a mean figure of 1033 kpm/min (S D=206 kpm/min, S E=36 kpm/min) was obtained, and for the women 625 kpm/min (S D=140 kpm/min, S E=27 kpm/min).

Patients with a chronic disease in addition to bronchial asthma

The 12 patients (6 men and 6 women) who were diagnosed as having an additional chronic disease — which it was considered might have had a decisive influence on the cardio-respiratory functions — are reported separately. Since these patients were relatively small in number and no significant differences were found between the sex and age groups among them, these factors are disregarded in the presentation of the results.

For the distribution of these patients into groups according to the estimated total number of sickness points for bronchial asthma since the onset of this disease, reference may be made to Table 23, page 62.

The mean value of W_{130} for these patients, as a percentage of the predicted value, was 53.0 (range 23.1—81.6 %). The corresponding mean value for W_{max} was 54.3 % (range 23.1—80.3). In calculating the predicted value regard was taken of sex and age. The calculations were based on the values for 10 out of the 12 patients.

The physical work capacity for this group of patients was on an average somewhat lower than for the rest of the asthma series, which is evident by comparison with the data given in Tables 21 and 22.

To summarize it may be said that both for W_{130} and W_{max} the observed mean value was less than the predicted value for all groups distributed according to sex, age and different case-history factors. For several of these groups the difference between the mean observed value and the predicted value was significant.

Some significant differences were also found between the mean observed values for the sub-groups of certain case-history factor classifications. As a rule however these differences did not reach the significance level.

With regard to W_{130}, significant differences were shown between certain sub-groups classified according to the estimated total number of sickness points and according to age at onset.

With regard to W_{max} significant differences were shown between certain sub-groups classified according to the estimated total number of sickness points, estimated number of attack hours, estimated mean number of sickness points per year during the five-year period 1957—61 and age.

For both W_{130} and W_{max} a significant relationship was shown with both the vital capacity and the lung function variables measuring ventilation capacity.

Neither for W_{130} nor W_{max} was any relationship found with the ratio of residual volume to total lung capacity.

Other function variables determined in connection with the submaximal work test

The results for some other variables studied on testing physical work capacity (Tables 31 and 32) will be given briefly below. These variables are the heart rate reaction to the orthostatic test, the maximum heart rate and maximum respiratory frequency during the work test and the lactate concentration in the blood immediately after the end of the work test. The mean value for each of these variables is given both for the male and the female patients. Two comparisons were made for each of the variables, firstly between groups classified according to sex, age and estimated total number of sickness points, and secondly between the control material and the corresponding sex and age groups in the asthma series. For these comparisons it will only be stated whether any significant difference was found, either between the groups in the asthma series or between the control material and the corresponding asthma patients.

In the orthostatic test the mean increase in heart rate in the men of the asthma series was found to be 13 beats/min and in the women 14 beats/min. This is a normal finding.

The highest mean heart rate attained during the work test was 161 beats/min in the men and 160 beats/min in the women; this reduced with increasing age and vice versa.

The highest mean respiratory frequency attained during the work test was 31 breaths/min in the men and 30 breaths/min in the women. This is a normal finding.

The mean lactate concentration in the blood immediately after the end of the work test was 7.2 mE/l in the men and 6.9 mE/l in the women. This meant that the work test did not en

percentage of predicted value at										
heart rate 170 beats/min			maximum performed work							
No.	kpm/min	% of patients at steady state	No.	kpm/min	% of pred.	% of patients at steady state	No	Heart rate (max.) beats/min	Resp rate (max.) breaths/min	Blood lact. mE/l
3	1013	33	3	1100	100 6	33	3	179	35	8.2
2	925	50	6	783	85 2	67	6	151	31	7.3
1	920	100	2	730	102 9	50	2	163	38	7.9
6	918	50	11	863	92.6	55	11	169	32	7 7
	1270			1600	125.5			190	40	11.1
	540			500	55.8			126	22	4.0
	301			321	23 5			19	6	2.4
	123 1			97.6	7 7			5.8	1.8	0 7
5	912	80	5	900	82.1	80	5	173	28	7 4
1	820	100	2	600	65.9	50	2	158	28	8.1
5	812	100	9	623	81.9	80	9	166	31	7.8
11	851	91	16	706	80.1	81	16	162	30	7 7
	1170			900	118.1			178	36	13 3
	620			300	41.9			131	21	3.6
	146			211	20.3			13	4	2.8
	44 1			52.8	5 1			3.3	1.0	0.7
4	920	50	5	900	75.3	60	5	168	29	8.1
2	725	50	4	525	58 9	50	4	169	32	6.5
3	933	0	5	600	63.7	20	5	152	31	7.5
9	851	33	14	707	73.9	43	14	160	32	8 1
	1230			1200	118.1			183	40	14.3
	700			300	35 8			122	22	2.8
	150			279	27 4			22	6	2.7
	53.1			74 5	7.3			5 9	1 6	0.7
12	852	56	13	918	83 7	62	13	173	38	6.7
5	784	60	12	667	73.7	58	12	15_	31	7.2
9	831	67	16	650	85 1	63	16	153	33	7 7
26	878	62	41	740	81 3	61	41	161	31	7.2
	1270			1600	125.5			190	40	14.2
	540			300	35 8			122	22	2.3
	168			265	23 5			18	5	2.7
	30.9			41.9	4 6			2 6	0.8	0 4

Groups acc. to sickness points and age (yrs.)		Characteristic	Heart rate, beats/min			Work load, kpm/min						
						heart rate 130 beats/min				heart rate 150 beats/min		
			No	rest	standing	No	kpm/min	% of pred.	% of patients at steady state	No	kpm/min	% of patients at steady state
A	30—49	mean	3	6	81	3	607	93.4	100	3	810	[illegible]
	50—59	„	0	69	83	6	632	97.2	83	4	835	[illegible]
	60—68	„	2	73	99	2	520	80.0	50	2	700	[illegible]
	Total A	mean	11	69	84	11	603	93.0	82	9	798	[illegible]
	„	highest		80	120		850	130.8			1060	
	„	lowest		50	54		250	38.5			420	
	„	SD		10.5	16.6		212	31.2			244	
	„	SE		3.2	5.0		61 1	9 4			81.5	
B	30—49	mean	5	70	84	5	538	62.8	60	5	724	[illegible]
	50—59	„	2	73	110	2	450	69.2	50	2	600	[illegible]
	60—68		9	6	88	9	458	0.5	8	8	658	[illegible]
	Total B	mean	16	6	88	16	482	74 1	75	15	672	[illegible]
	„	highest		104	116		790	121.5			970	
	„	lowest		52	62		240	36.9			430	
		SD		15.5	15.0		147	22.6			153	
	„	SE		3.9	3.8		36.7	5.7			39 4	
C	30—49	mean	5	69	79	5	582	89.5	80	5	64	[illegible]
	50—59		4	83	96	4	400	61.5	75	3	597	[illegible]
	60—68		5	60	83	5	531	82.2	40	3	783	
	Total C	mean	14	72	86	14	513	78.9	64	11	724	[illegible]
	„	highest		92	110		020	141.5			1070	
	„	lowest		48	62		250	38.5			450	
		SD		12.8	12.6		164	25.3			156	
	„	SE		3.4	3 4		43.0	6.8			47.0	
Total A+B+C	30—49	mean	13	71	81	13	571	87 9	85	13	759	6
	50—59		12	74	90	12	524	80.6	75	9	703	5
	60—68		16	73	88	16	490	75.4	63	13	693	6
	30—68	mean	41	73	86	41	526	80.8	73	35	721	6
		highest		104	120		920	141.5			1070	
		lowest		48	54		240	36.9			420	
		SD		13.5	14 4		174	26.5			183	
		SE		2.1	2.3		27.2	4.1			30 9	

Tabl 31 *Observati ns connected with the submaximal work test in mal patients*

…entage of predicted value t										
…eart rate 170 beats/min			maximum performed work					Heart rate (max.) beats/min	Resp rate (max.) breaths/min	Blood lact. mE/l
…s.	kpm/min	% of patients in steady state	No.	kpm/min	% of pred.	% of patients in steady state	No.			
1	1013	33	3	1100	100.6	33	3	179	26	8.3
[illegible]	925	50	6	783	85.2	67	6	161	31	7.3
1	820	100	2	750	102.8	50	2	163	25	7.9
[illegible]	918	50	11	863	97.6	53	11	169	27	7.7
	1370			1300	125.4			190	40	11.1
	540			500	53.8			126	22	4.0
	301			321	25.5			19	6	2.4
	123.1			97.6	7.7			4.6	1.8	0.7
5	912	80	5	900	83.1	80	5	173	25	7.4
1	620	100	2	600	66.9	50	2	158	28	8.1
5	617	100	9	622	81.9	80	9	156	31	7.8
11	834	91	16	706	80.1	81	16	162	30	7.7
	1170			900	118.1			178	36	12.3
	620			300	41.8			134	21	3.8
	140			211	20.3			12	4	2.6
	41.1			52.8	5.1			3.3	1.0	0.7
4	920	50	5	900	73.2	60	5	168	28	8.1
2	725	50	4	623	50.9	50	4	150	27	6.8
3	930	0	5	600	83.7	20	5	152	31	7.5
9	851	33	14	707	72.8	43	14	160	32	6.4
	1330			1300	118.1			185	40	14.2
	700			300	25.8			122	22	2.3
	159			279	27.4			22	6	2.7
	53.1			74.5	7.3			5.9	1.6	0.7
12	932	58	13	916	83.7	62	13	173	28	6.7
5	781	80	12	867	73.7	58	12	165	31	7.3
9	831	67	16	650	83.1	63	16	155	33	7.7
26	872	63	41	749	81.3	61	41	161	31	7.3
	1370			1300	123.6			190	40	14.9
	340			300	33.6			122	22	2.3
	148			288	25.6			18	5	2.7
	36.9			41.9	4.0			2.8	0.5	0.4

Groups acc. to sickness points and age (yrs)		Charac teristic	Heart rate beats/min			Work load,					
						heart rate 130 beats/min				heart rate 1[illegible]	
			No	rest	standing	No	kpm/ min	% of pred.	% of patients at steady state	No.	kpm/ min
A	30—49	mean	12	79	94	12	323	78.6	67	12	433
	50—59		5	78	90	5	308	89.5	60	5	494
	60—68		8	0	91	8	340	84.2	50	5	354
	Total A	mean	25	78	93	25	339	82.6	60	22	439
	"	highest		98	118		680	165.3			660
	"	lowest		54	72		150	36.5			230
		SD		10 7	13.8		127	30.9			121
	"	SE		2..	2.8		25 4	6.2			25.[illegible]
B	30—49	mean	11	77	90	11	326	79 3	64	11	465
	50—59		7	80	101	6	253	01.3	83	6	402
	60—68		7	81	91	7	377	91 7	57	6	493
	Total B	mean	.5	70	93	24	322	8.5	67	23	456
	"	highest		110	134		5?0	126.5			660
		l west		58	66		1?0	30.2			2?0
	"	SD		15.5	18.1		125	30.5			116
	"	SE		3 0	3.0		25.6	6.2			24.2
C	30—49	mean	6	83	96	6	?68	65.2	67	6	393
	50—59		5	81	93	4	2 0	65 7	50	3	420
	60—68		12	79	92	11	301	73.2	64	6	406
	Total C	mean	23	81	93	21	286	69.5	62	15	404
		highest		104	115		500	121 7			500
	"	lowe t		61	72		180	43.8			260
	"	SD		12.0	10 1		94	22.4			95
		SI		2.6	2.2		20 6	4 9			21.3
Total A+B+C	30—49	mean	29	70	93	20	313	6.3	66	29	430
	50—59		17	80	95	15	206	72.0	67	14	439
	60—68		27	79	92	26	335	81.5	58	17	430
	30—68	mean	73	79	93	70	318	77.3	03	60	437
		highest		110	134		680	165.5			660
	"	lowest		54	66		120	30.2			230
		SD		12.9	14.5		118	28.6			113
		SE		1.5	1 7		14.2	3.4			14.8

Table 32 *Observation conn cted with the bmaximal work test in female patients.*

percentage of predicted value t										
heart rate 1˚0 beats/min			maximum perf rmed work							
No.	kpm/min	% of patients t steady state	No.	kpm/min	% of pred.	% of patient t steady state	No.	Heart rat (max.) beats/min	Resp rate (max.) breaths/min	Blood lact. mE/l
9	337	56	12	530	72.5	50	12	174	28	6.8
2	640	50	5	480	87.7	60	5	154	33	5.8
3	467	0	8	330	83.6	50	8	141	30	5.2
14	350	43	25	472	79.1	52	25	160	30	6.1
	800			800	136.3			190	40	9.7
	150			200	40.0			116	23	3.1
	121			161	21.9			20	5	2.5
	22.9			30.3	4.4			4.0	0.9	0.5
9	584	56	11	545	74.5	61	11	167	25	7.3
4	520	75	7	407	78.4	86	7	149	29	7.8
2	615	50	7	479	101.6	87	7	148	31	7.0
15	571	60	25	474	83.2	68	25	157	29	7.3
	780			800	151.1			180	40	12.6
	280			200	36.8			104	20	3.1
	116			133	26.6			18	5	2.4
	30.0			26.6	5.3			3.6	1.0	0.5
6	317	33	6	400	68.5	33	6	172	29	8.1
2	640	0	5	290	82.6	40	5	142	28	7.6
3	520	67	12	333	77.5	75	12	143	32	6.5
11	522	36	23	367	69.7	57	23	160	30	7.1
	50			600	121.9			185	40	11.4
	310			80	9.0			98	15	2.7
	140			154	28.4			23	6	2.2
	42.4			37.2	5.9			5.5	1.3	0.5
24	337	60	20	538	72.4	52	20	171	28	7.3
8	335	50	17	384	72.3	65	17	148	31	7.1
8	524	38	27	353	86.3	63	27	145	31	8.3
40	560	48	73	410	77.5	60	73	156	30	8.9
	800			800	151.6			190	40	12.6
	310			80	9.0			98	18	2.7
	122			152	26.0			22	5	2.2
	19.8			17.9	3.0			3.6	0.6	0.3

HEMODYNAMIC STUDIES

Right heart catheterization was performed without complications on 69 out of a total of 78 patients who had not reached the age of 60 years

The remaining nine patients were unable to go into hospital for the catheterization, for various private reasons (e g a young mother with small children an employer who was not able to leave his firm) These patients did take part, however in the tests of lung function and physical work capacity that could be carried out in the out-patients department, and some idea of their cardio-respiratory function was still therefore obtained

The distribution of the nine patients into groups according to their sickness points (see page 25) is shown in Table 33 This table also shows the corresponding distribution of all the 72 patients who had not reached the age of 60 years. (The six patients with another chronic disease besides the bronchial asthma, which it was considered might have had decisive influence on the different functions, were excluded)

The table shows that in this group of nine patients the estimated degree of severity of the asthma — as judged by the total number of sickness points — had been relatively mild None of the patients were assigned to group C (see page 35) and no fewer than seven of the nine were assigned to group A. The mean number of sickness points for these patients was 684 while that for the entire material was 1587

Table 34 shows the mean values and ranges for ventilation capacity lung volumes and physical work capacity as a percentage of the predicted value. Tests of physical work capacity ventilation capacity and vital capacity were performed on all nine patients, while investigations of the other lung volumes were performed on five patients. For comparison, the mean values for the material as a whole are given in parenthesis in the table these values are taken from Table 19, page 58.

Groups according to sickness points	No	Sickness points mean
A (<1000 p)	7 (27)	488
B (1000—1999 p.)	2 (26)	1380
C (≧2000 p)	0 (19)	—
Total	9 (72)	684 (1587)

Table 33 *Distribution into groups according to estimated number of sickness points, for 9 patients below the age of 60 years who did not undergo heart catheterization. The corresponding figure for all of the 72 patients in the material who had not reached the age of 60 years, is given in parenthesis.*

	VC % of pred.	FEV1.0 % of pred	FEV% of pred.	MVV40 % of pred	MVVF % of pred.
mean	93.7 (89.8)	86.9 (72.0)	91.8 (79.5)	79.4 (64 1)	80 1 (63 2)
range	88.2—103 0	73.9—100.0	81 7—101.5	70 0—96.3	72 0—102.0

	TLC % of pred.	FRC/TLC % of pred	RV/TLC % of pred.	W130 % of pred.	Wmax % of pred.
mean	83.1 (93.8)	97 0 (115.1)	113 0 (115.1)	83.0 (78.1)	83.2 [illegible] 4 6)
range	71 4—92.9	78.7—118.8	89 0—131.2	36 5—114 4	60 6—136.3

Table 34 *Ventilation capacity lung volumes and physical work capacity as a percentage of the predicted value for patients who did not undergo heart catheterization. The corresponding mean figure for the whole group of patients who had not reached the age of 60 years is given in parenthesis.*

The tables concerning both the degree of severity of the asthma and the lung function and physical work capacity show that these nine patients, regarded as a group differed from the rest of the material in that their values for the above variables were on an average more favourable with the exception of the mean observed value for the total lung capacity which was somewhat lower than the corresponding value for the rest of the series.

Of the 69 patients who underwent heart catheterization, six had another chronic disease apart from the bronchial asthma (see page 160) which it was considered might have had a decisive influence on the cardio-respiratory functions. These patients were therefore not included in the analyses of the data obtained in the hemodynamic studies, but are reported separately (page 88, Table 36) The remaining 63 patients, 25 men and 38 women, thu constituted the material on which the analyses were based.

Sinc however for one f the men and ne of the women alues of 200 and 100 kpm/min espectivel were obtained at the first load in the work test compared with 300 nd 200 for the the men and women, these tw patient were luded from the work test analyses They are however represented in the Figures showing the mutual relationships f the ables For neither of these patient were the hemodynamic lues found to differ to ny notable ext t from the mea lue for the group with regard to any of the different ariable studied in the hemodynamic investigations

Results

The observations made during the heart catheterization, both during an ordinary resting state and during anoxic anoxia and the graded work test, are given in Tables 37—44 pages 90—97

The *right atrial pressure* was found to be normal in all patients during a resting state (Tables 37—38)

The mean value for the *systolic pressure* at rest (Tables 37—38) in the *pulmonary artery* was normal (normal values as given by Holmgren, Jonsson and Sjöstrand, 1960) i. e. 19 mm Hg both for the men and the women. The highest pressure recorded was 31 mm Hg (Table 37) During the graded work load test (Tables 41—44) an almost d ul led systolic pressure was on an average observed. During standardized anoxic anoxia (Tables 39—40) the pressure in the men rose, on an a erage, to 31 mm Hg from 19 mm Hg at a normal resting state. The corresponding values for the women were 27 and 19 mm Hg respectively For both sexes the increase during anoxic anoxia was significant (***)

When the patients were classified into groups according to the estimated total number of ickness points, no noteworthy difference with regard to the systolic pressure was found between these groups either during a normal resting state, work load or anoxic anoxia.

The average value for the *mean pressure* at rest in the *pulmonary artery* was normal, i. e. 12 mm Hg for both men and women The highest value measured was 20 mm Hg and this also lay within the normal limits. During the work load test an almost doubled mean pressure was on an average observed.

During anoxic anoxia the mean

pressure in the men rose on an average to 10 mm Hg from 12 mm Hg at a normal resting state. The corresponding *values for* the women were 17 and 12 mm Hg respectively. The increase during anoxic anoxia was significant for both sexes (***). When the patients were classified into groups according *to the estimated total* number of sickness points a mean pressure of 30 mm Hg was *obtained for the four men* of ages 50—59 years and who had more than 2000 points, while the corresponding pressure for the eight men in the same age group who had not *attained 2000 points* was *10.5 mm Hg*. This difference might be due to the fact that two of the four men who had attained more than 2000 points had about 7700 and 3300 points and a disease duration of 50 and 37 years respectively. Their mean pressures in the pulmonary artery were 47 and 44 mm Hg respectively. The other two men had only a few more than 2000 sickness points their pressures lay even during anoxic anoxia within the normal variation for the mean pressure in the pulmonary artery during a normal *resting state*.

No other noteworthy differences were found on classification of the *patients into groups according to sex*, age and sickness points, either during a normal resting state, work load or anoxic anoxia. No significant pressure gradients over the pulmonary valve were *demonstrated*.

The mean value for the *diastolic pressure* at rest in the *pulmonary* artery was ial i e. 7 mm Hg for the men an l mm for the women.

The *pulmonary capillary venous* (PCV) *mean pressure* at rest lay within the normal variation in all patients. The highest pressure recorded was 11 mm Hg. In one patient a pressure of 15 mm Hg was observed during exercise. *Disregarding this patient* the highest observed value during work was 13 mm Hg. During exercise the mean value for the mean PCV pressures increased somewhat, but the *difference on comparison* with the corresponding value for the PCV pressure during a resting state was not significant.

The *pulmonary vascular resistance (R)* expressed as *mm Hg per l/min*

$$\frac{\bar{P}_{PA}-\bar{P}_{PCV}}{\dot{Q}}$$

was determined at rest and during exercise (Tables 37—38 41—44 and Figure 7). The pulmonary vascular resistance index (R_I) expressed as mm Hg per l/min m² BSA,

$$\frac{\bar{P}_{PA}-\bar{P}_{PCV}}{\dot{Q}}$$

BSA, was also calculated for the same patients. The mean pulmonary vascular resistance at rest for the men was 1 40 and for the women 1 41. On classification of the *material into groups* according to the estimated total number *of* sickness points no significant difference was found between these groups. Neither was there any significant difference between the pulmonary vascular resistance at rest and *that during* work.

The average systolic *brachial pressure* during a state of rest was 135 mm Hg for the men of ages 30—49 years and 151 mm *Hg for* those of ages 50—59 years. The corresponding values for the women were 130 and

138 mm Hg. The highest value observed among the men was 1 0 and among the women 205 mm Hg.

The average mean and diastolic brachial pressure at rest for the men of ages 30—49 years were 100 and 76 mm Hg respectively and for the men of ages 50—59 years 112 and 83 mm Hg respectively. The corresponding values for the women were 101 and 78 and 114 and 80 mm Hg, respectively.

In no subject were abnormally high or low arterial blood pressure changes observed during exercise.

On an average the *oxygen uptake* during heart catheterization was not significantly higher (3.2 %) than under BMR conditions, and was on an average 6.8 % higher than the predicted basal value at rest. (Predicted from sex, age, body height and body weight according to Harris and Benedict, 1919). During anoxic anoxia the oxygen uptake was on an average 9 % lower than during normal resting conditions in connection with the catheterization.

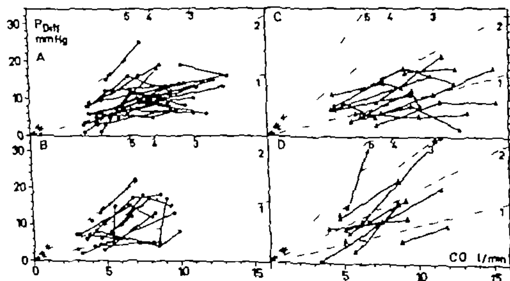


Fig. 7. The relationship between pressure gradient (P_{Diff}) over pulmonary vascular bed — expressed as the difference between pulmonary arterial mean pressure and pulmonary capillary venous mean pressure in mm Hg — and cardiac output (CO), l/min.

The interrupted lines represent different pulmonary vascular resistance (R). Circles represent females, triangles males. Open symbols represent the condition during rest. Symbols with dot represent the condition during work, 300 kpm/min for females and 300 kpm/min for males. Filled symbols represent the condition during work, 400 kpm/min for females and 600 kpm/min for males.

Fig. 7 A shows the conditions for females 30—49 years old, Fig. 7 B for females 50—59 years old, Fig. 7 C shows the conditions for males 30—49 years old, Fig. 7 D for males 50—59 years old. For one symbol there was no room in Fig. 7 D. This symbol (see arrow in the figure) represents R = 6.2 (P_{Diff} = 41 mm Hg; CO = 8.6 l/min).

pressure in the men rose on an average to 19 mm Hg from 12 mm Hg at a normal resting state. The corresponding values for the women were 17 and 12 mm Hg respectively The increase during anoxic anoxia was significant for both sexes (***) When the patients were classified into groups according to the estimated total number of sickness points a mean pressure of 30 mm Hg was obtained for the four men of ages 50—59 years and who had more than 2000 points, while the corresponding pressure for the eight men in the same age group who had not attained 2000 points, was 19.5 mm Hg This difference might be due to the fact that two of the four men who had attained more than 2000 points had about 7700 and 3300 points and a disease duration of 50 and 37 years respectively Their mean pressures in the pulmonary artery were 47 and 44 mm Hg respectively The other two men had only a few more than 2000 sickness points their pressures lay even during anoxic anoxia within the normal variation for the mean pressure in the pulmonary artery during a normal resting state.

No other noteworthy differences were found on classification of the patients into groups according to sex, age and sickness points either during a normal resting state, work load or anoxic anoxia. No significant pressure gradients over the pulmonary valve were demonstrated.

The mean value for the *diastolic pressure* at rest in the *pulmonary* artery was normal i e. 7 mm Hg for the men and 8 mm for the women.

The *pulmonary capillary venous* (PCV) *mean pressure* at rest lay within the normal variation in all patients. The highest pressure recorded was 11 mm Hg In one patient a pressure of 15 mm Hg was observed *during exercise* Disregarding this patient the highest observed value during work was 13 mm Hg During exercise the mean value for the mean PCV pressures increased somewhat but the difference on comparison with the corresponding value for the PCV pressure during a resting state was not significant

The *pulmonary vascular resistance* (R) expressed as mm Hg per l/min $\frac{\overline{P}_{PA}-\overline{P}_{PCV}}{\dot{Q}}$ was determined at rest and during exercise (Tables 37—38, 41—44 and Figure 7) The pulmonary vascular resistance index (R_I) expressed as mm Hg per l/min m^2 BSA $\frac{\overline{P}_{PA}-\overline{P}_{PCV}}{\dot{Q}}$ BSA, was also calculated for the same patients The mean pulmonary vascular resistance at rest for the men was 1 49 and for the women 1 41 On classification of the material into groups according to the estimated total number of sickness points no significant difference was found between these groups Neither was there any significant difference between the pulmonary vascular resistance at rest and that during work

The average systolic *brachial pressure* during a state of rest was *135* mm Hg for the men of ages 30—49 years and *151 mm Hg for those of ages 50—59 years The corresponding values for the women were 139 and*

ant difference was found between different groups of the material on classification according to sickness points.

The mean *oxygen saturation of mixed venous blood* (drawn from the pulmonary artery) was 71.3 % (range 60.6—81.4) at rest. When the material was divided into groups according to sex, age and estimated total number of sickness points, no significant difference between these groups was found. During exercise the saturation decreased with increasing working intensity and heart rate (Figure 8). The lowest value observed was 24.0 %.

The mean *arterio-venous oxygen difference* (AVD ml/l) was 45.2 ml/l (range 29.7—59.7) at rest. When the material was divided into groups according to sex and estimated total number of sickness points no significant difference between these groups was found.

During anoxic anoxia the arterio-venous oxygen difference decreased by an average of 3.3 ml/l to an average

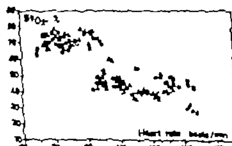


Fig. Oxygen saturation of blood in the pulmonary artery (S) per cent, in relation to heart rate beats/min at rest and during work.

Circles represent females and triangles males. Open symbol represent patient 30—49 years old, filled symbols patient 50—59 years old.

of 41.9 ml/l (range 27.1—64.2). When the material was divided into groups according to sex and sickness points, no significant difference between the different groups was shown for this value during anoxic anoxia.

During muscular work the AVD values increased with increasing working intensity. The highest values observed for the men were 111.2 ml/l at 300 kpm/min and 133.4 ml at 600 kpm/min. The corresponding figures for the women were 104.2 ml/l and 118.9 ml/l at 200 and 400 kpm/min respectively. In Figure 9 AVD is plotted against oxygen uptake, and in Figure 10 against heart rate at rest and during exercise.

Both for the resting state, anoxic anoxia and for the graded work test, the mean value for the AVD in the older age group was somewhat higher than in the younger age group.

The mean *cardiac output* at rest was 5.6 l/min for the men of ages 30—49 years and 4.6 for the men of ages 50—59 years. The corresponding cardiac indices were 3.0 and 2.7 respectively. For the women of ages 30—49 years the mean minute volume was 5.1 litres and for those of ages 50—59 years 4.2 litres. The corresponding cardiac indices were 3.0 and 2.5 respectively. On classification of the material into groups according to the estimated total number of sickness points, no noteworthy difference was observed between the groups.

During anoxic anoxia the cardiac minute volume increased for the men — in comparison with that during a resting state — by an average of 0.3 litre while for the women there was

During exercise the oxygen uptake increased with increasing work intensity. The mean mechanical efficiency values were 21.0 % and 23.6 % for the men at 300 kpm/min and at 600 kpm/min respectively. The corresponding values for the women at 200 and 400 kpm/min were 19.7 % and 23.1 % respectively. For the entire material regardless of sex and graded load the efficiency value was 22.6.

A comparison between the rate of work and heart rate in the sitting position without cardiac catheterization and in the supine position with cardiac catheterization was made.

The difference between the working capacity in the sitting and supine positions was determined for both the heart rates 130 and 150. In 54 patients, regardless of sex, the W_{130} value in the supine position was 403 kpm/min, i. e. 16 kpm/min higher on an average than that in the sitting position. At the higher heart rate level the W_{150} value in the supine position was 406 kpm/min, i. e. 13 kpm/min higher on an average than that in the sitting position (30 patients). Neither for W_{130} nor W_{150} was the difference between the sitting and supine position significant.

The difference in heart rate between the sitting and supine positions at a certain given load was studied. A mean heart rate of 106 beats/min was recorded for 23 men during exercise at 300 kpm/min in the sitting position and 107 beats/min during similar exercise in the supine position. The corresponding value for 10 of the men during exercise at 600 kpm/min was 135 beats/min both in the sitting and supine positions. For 33 women a mean heart rate of 116 beats/min was recorded during exercise of 200 kpm/min in the sitting position and 113 beats/min during similar exercise in the supine position. The corresponding values for 20 of the women during exercise of 400 kpm/min were 146 and 141 beats/min, respectively. Neither for the men nor for the women was the difference in heart rate between the sitting and supine positions at different work loads of a noteworthy order of size.

The mean arterial oxygen saturation at rest was 96.4 % (range 87.0—100.0). When the material was divided into groups according to sex, age and estimated total number of sickness points, no significant difference between the different groups was found, with regard to this value.

During anoxic anoxia the arterial oxygen saturation decreased for both sexes by an average of 20 per cent units to an average of 76.3 % (range 53.4—95.1). On classification of the material into groups according to age and sickness points, no significant difference between these groups was found for this value during anoxic anoxia.

During exercise of 300 kpm/min for men and 200 kpm/min for women the mean arterial oxygen saturation decreased somewhat, i. e. from an average of 96.0 % during a resting state to 95.0 %, and from 96.7 % to 96.1 % respectively; these differences were not however significant. Neither was there any significant difference for the men and women who performed the second work load test, i. e. at 600 and 400 kpm/min respectively on comparison with the respective mean values for the arterial oxygen saturation during a resting state. No signific

ant difference was found between different groups of the material on classification according to sickness points.

The mean *oxygen saturation of mixed venous blood* (drawn from the pulmonary artery) was 71.3 % (range 60.6—81.4) at rest. When the material was divided into groups according to sex, age and estimated total number of sickness points, no significant difference between these groups was found. During exercise the saturation decreased with increasing working intensity and heart rate (Figure 8). The lowest value observed was 24.0 %.

The mean *arterio-venous oxygen difference* (AVD ml/l) was 45.2 ml/l (range 29 —59.7) at rest. When the material was divided into groups according to sex and estimated total number of sickness points no significant difference between these groups was found.

During anoxic anoxia the arterio-venous oxygen difference decreased by an average of 3.3 ml/l to an average of 41.9 ml/l (range 27.7—64.2). When the material was divided into groups according to sex and sickness points, no significant difference between the different groups was shown for this value during anoxic anoxia.

During muscular work the AVD values increased with increasing working intensity. The highest values observed for the men were 111.2 ml/l at 300 kpm/min and 133.4 ml at 600 kpm/min. The corresponding figures for the women were 104.2 ml/l and 118.9 ml/l at 200 and 400 kpm/min respectively. In Figure 9 AVD is plotted against oxygen uptake, and in Figure 10 against heart rate at rest and during exercise.

Both for the resting state, anoxic anoxia and for the graded work test, the mean value for the AVD in the older age group was somewhat higher than in the younger age group.

The mean *cardiac output* at rest was 5.6 l/min for the men of ages 30—49 years and 4.8 for the men of ages 50—59 years. The corresponding cardiac indices were 3.0 and 2.7 respectively. For the women of ages 30—49 years the mean minute volume was 5.1 litres and for those of ages 50—59 years 4.2 litres. The corresponding cardiac indices were 3.0 and 2.5 respectively. On classification of the material into groups according to the estimated total number of sickness points, no noteworthy difference was observed between the groups.

During anoxic anoxia the cardiac minute volume increased for the men — in comparison with that during a resting state — by an average of 0.3 litre, while for the women there was

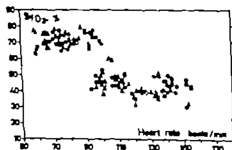


Fig. 8. Oxygen saturation of blood in the pulmonary artery ($S_{\bar{v}O_2}$), per cent, in relation to heart rate beats/min at rest and during work.

Circles represent females and triangles males. Open symbols represent patients 30—49 years old, filled symbols patients 50—59 years old.

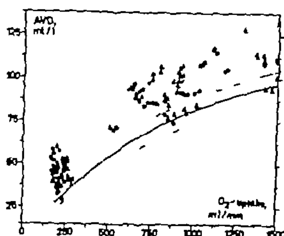


Fig 9 Arterio-venous oxygen difference (AVD) ml/l in relati n to oxygen uptake ml/min STPD at rest and during work

Central line indicates regression between these parameters in a control material In t rrupted lines indicate ± standard error of estimate. The control material has been collected by Holmgren (personal communica tion) from results published by Holmgren et al (1957 1960) and Bevegård et al. (1960)

Symbols as in Fig 8.

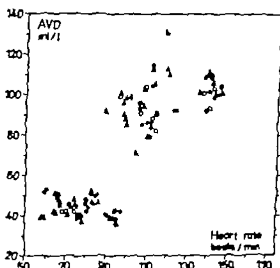


Fig 10 Arterio-venou xygen difference (AVD) ml l in relation to heart ra beats/ min, at est and d ing wo k

Symbol s in Fig 8.

an average decrease of 0 4 litre. For neither sex was the difference signific ant. When the material was divided into groups according to the sickness points, no significant difference be tween these groups was found

During exercise at 300 kpm/min for the men and 200 kpm/min for the women, the cardiac minute volumes were on an average 9.2 and 8 0 litres respectively For those men and wom en who performed the second work load test i e 600 kpm/min and 400 kpm/min respectively the cardiac mi nute volumes increased on an average to 12 7 and 10 1 litres The relation of the cardiac minute volume to the oxy gen uptake at rest and during exercise is shown in Figure 11 On classifica tion of the material into sickness point groups no significant difference with regard to the minute volume was shown between these groups.

The mean *stroke volume* at rest was 72 ml for the men and 61 ml for the women On classification of the mater ial into sickness point groups no noteworthy difference was found be tween these groups

The stroke volume during anoxic anoxia decreased in comparison with that during a resting state by an aver age of 6 ml for the men and 10 ml for the women For neither sex however was the difference significant Neither was there any significant difference when the material was divided into groups according to the number of sickness points.

In the men during exercise at 300 kpm/min and in the women during exercise at 200 kpm/min, the stroke volume increased — in comparison

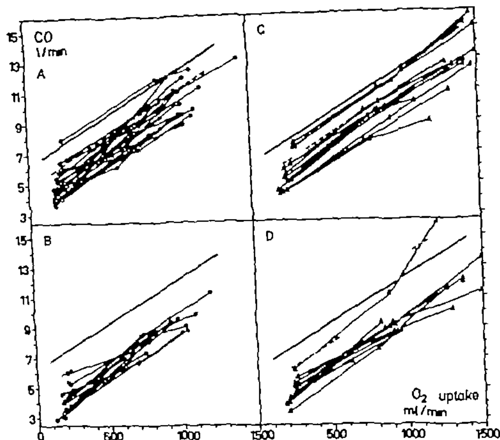


Fig 11 Oxygen ptak ml min, STPD i relation t cardiac output l/min, at rest and during work

Central lines indicat regression between these parameters in control material Inter rupted lines indicat ± standard error of estimate The control material has been ollected by Holmgren (personal communication) from esult published by Holmgren et l. (1957 1960) nd Bevegård et al. (1960)

Fig 11 A hows the conditions for females 20–49 yea old, Fig. 11 B *for females 40–49* years old. Fig 11 C shows the conditions for males 20–49 years old, Fig. 11 D for males 50–59 years old Symbols in Fig 7 For one symbol there was no room in Fig. 11 D. This symbol (see rrow in the figure) represent cardiac output = 19.8 l/min nd xygen uptak = 1460 ml/min STPD

with that during a resting state — by an verage of 18 ml and 10 ml to 90 ml and 71 ml respectively This increase was significant for both sexes (   )

For the men and women who per formed the second work load test, i e. 600 and 400 kpm/min respectively no noteworthy change in the stroke vol-

ume was found on comparison with the first load. For the men there was a mean increase in stroke volume of 4 ml and for the women a mean decrease of 1 ml When however the men were classified into sickness point groups it was found that for those who had fewer than 1000 points (group A) the stroke volume during exercise at 300 kpm/min was on an average 10 ml higher than that at rest and the stroke volume during exercise at 600 kpm/min was on an average 27 ml higher than the resting value The corresponding increases for the men who attained 2000 points or more (group C) were 12 and 15 ml respectively The difference between the increase in stroke volume for group A and that for group C was not however significant For the women, no noteworthy difference was found between the different sickness point groups. The relation between stroke volume/blood volume and heart rate is shown in Figure 12

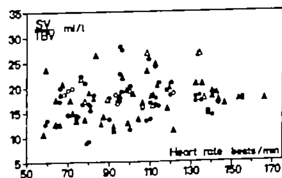


Fig 1 Stroke volume (SV) divided by total blood volume (TBV) ml/l in relation to heart rate beats/min at rest and during work

Symbols as in Fig 8.

The influence of different case history factors on the variables used for measuring hemodynamic or respiratory function

For each of the following variables
mean pressure in the pulmonary artery during exercise,
mean pressure in the pulmonary artery during anoxic anoxia
arterial oxygen saturation during exercise,
arterial oxygen saturation during anoxic anoxia,
pulmonary vascular resistance during exercise
forced expiratory volume during one second at rest
ratio of residual volume to total lung capacity

a selection was made from 56 patients i e those men and women who during heart catheterization performed exercise at 300 and 200 kpm/min respectively In order to obtain the best contrast between two groups of patients, a selection was made from the 56 patients of firstly the 20 who showed the greatest pathological deviation from normal values, and secondly the 20 who deviated most greatly in the opposite direction

The aim was to study for each of the variables, the difference between these two groups of patients with regard to each of the case-history factors given on page 41

In neither group of patients did the observed ratio of men/women differ significantly from the predicted No regard was therefore taken of sex in the subsequent analyses.

The estimated degree of influence of

the different case-history factors on each of the function variables will be given below

Mean pressure in the pulmonary artery during exercise

The 20 patients who were recorded a having the highest pressures had a mean value of 30 1 mm Hg (range 23—63) while the mean value of those with the lowest pressures was 14 7 mm Hg (range 8—17)

The difference between the two groups was found to be significant ( ) for age, age at onset and the estimated mean annual sickness points for the period 1957—61 Those with a higher age, those with a higher age at onset and those with a higher number of sickness points during this later 5-year period showed on an average a relatively high mean pressure in the pulmonary artery during exercise. No significant differences were found for the estimated total sickness points, duration of the asthma, estimated number of attack hours or number of admissions to hospital on account of asthma

Mean pressure in the pulmonary artery during anoxic anoxia

The 20 patients who were recorded as having the highest pressures had a mean value of 25 6 mm Hg (range 18—47) while the mean value of those with the lowest pressures was 11.8 mm Hg (range 9—14)

For none of the case-history factors studied here was there any significant difference between the two groups.

Arterial oxygen saturation during exercise

The mean value for the 20 patients for whom the lowest oxygen saturation values in the brachial artery were obtained was 92.4 % (range 82.0—93 0) The corresponding value for the 20 patients with the highest values was 98 4 % (range 97 6—99.9)

The difference between the two groups of patients was statistically significant ( ) for age and age at onset those patients with a higher age and those with a higher age at onset showed on an average a relatively low arterial oxygen saturation during exercise No significant difference was found for the other case-history factors.

Arterial oxygen saturation during anoxic anoxia

The 20 patients for whom the lowest oxygen saturation values in the brachial artery were obtained had a mean value of 66 7 % (range 53 4—75.3) The corresponding value for the 20 patients with the highest saturation values was 84 7 % (range 79 1—95 1)

The difference between the two groups of patients was found to be significant ( ) both for age and for the number of admissions to hospital on account of bronchial asthma. Those with a higher age and those with a higher number of hospital admissions (≥5) showed on an average a relatively low oxygen saturation value in the brachial artery during anoxic anoxia. No significant difference was found for the other case-history factors.

Pulmonary vascular resistance (R) during exercise

The 20 patients for whom the highest vascular resistance was recorded

ume was found on comparison with the first load For the men there was a mean increase in stroke volume of 4 ml and for the women a mean decrease of 1 ml When, however the men were classified into sickness point groups it was found that for those who had fewer than 1000 points (group A) the stroke volume during exercise at 300 kpm/min was on an average 10 ml higher than that at rest and the stroke volume during exercise at 600 kpm/min was on an average 27 ml higher than the resting value The corresponding increases for the men who attained 2000 points or more (group C) were 12 and 15 ml respectively The difference between the increase in stroke volume for group A and that for group C was not however significant. For the women no noteworthy difference was found between the different sickness point groups The relation between stroke volume/blood volume and heart rate is shown in Figure 12

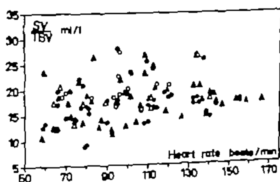


Fig 12 Stroke volume (SV) divided by total blood volume (TBV) ml/l in relation to heart rate beat min, at rest and during work

Symbols as in Fig 8.

The influence of different case history factors on the variables used for measuring hemodynamic or respiratory function

For each of the following variables mean pressure in the pulmonary artery during exercise
mean pressure in the pulmonary artery during anoxic anoxia
arterial oxygen saturation during exercise,
arterial oxygen saturation during anoxic anoxia
pulmonary vascular resistance during exercise,
forced expiratory volume during one second at rest
ratio of residual volume to total lung capacity

a selection was made from 56 patients i e those men and women who during heart catheterization performed exercise at 300 and 200 kpm/min respectively In order to obtain the best contrast between two groups of patients, a selection was made from the 56 patients of firstly the 20 who showed the greatest pathological deviation from normal values, and secondly the 20 who deviated most greatly in the opposite direction

The aim was to study for each of the variables, the difference between these two groups of patients with regard to each of the case-history factors given on page 41

In neither group of patients did the observed ratio of men/women differ significantly from the predicted. No regard was therefore taken of sex in the subsequent analyses.

The estimated degree of influence of

had a statistically significant influence. Other case-history factors with a similar degree of influence on one or more of the variables were the estimated mean annual number of sickness points during the five years almost immediately preceding the investigation, the estimated total number of attack hours, the number of admissions to hospital on account of bronchial asthma and the age at onset of the disease.

Study of the relationship between pairs of variables measuring hemodynamic function, ventilation capacity and lung volumes

A study was made of the relationship between different pairs of each of the variables mentioned on page 84 constituting a measure of hemodynamic function, ventilation capacity or lung volumes.

The material comprised 58 patients, i.e. those men and women who during the heart catheterization performed exercise at 300 and 200 kpm/min respectively. For each of the seven variables the 20 patients showing the least favourable values were selected. The number of patients among these 20 who were common to any two variables was then noted.

Assuming that no relationships existed, the predicted number of persons in each figure in Table 35 would be 7.1. As the table shows, there was a significant relationship (*) between the mean pressure in the pulmonary artery during relatively light muscular exercise and both the corresponding pressure during anoxic anoxia and the pulmonary vascular resistance during

	PA_m m.w	S_{aO_2} m.w	R m.w	PA_m a.	S_{aO_2} a.	$FEV_{1.0}$	RV/TLC
PA_m m.w		11	13	13*	13	9	7
S_{aO_2} m.w	11		12	11	12	11	8
R m.w	13*	12		11	11	9	10
PA	13*	11	11		15	10	6
S_{aO_2}	12	13	11	16		9	7
FEV	9	11	8	10	9		8
RV/TLC	7	9	10	6	7	8	

Table 35. For pairs of variables the number of patients out of the 20 showing the least favourable values of each of the variables. The degree of significance is asterisked.

PA_m m.w = mean pressure in pulmonary artery during light muscular work
PA_m a. = " during anoxic anoxia
S_{aO_2} m.w = oxygen saturation in brachial artery during light muscular work
S_{aO_2} a. = " during anoxic anoxia
R m.w = pulmonary vascular resistance during light muscular work
FEV = forced expiratory volume in one second at rest
RV/TLC = residual volume in relation to total lung capacity at rest

had a mean value of R=2 42 (range 1 67—6 28) The 20 patients with the lowest vascular resistance had a mean value of R=0 96 (range 0.50—1.33)

The difference between the two groups of patients was significant (*) both for age and for age at the onset of asthma Those with a higher age and those with a higher age at onset showed on an average a relatively high pulmonary vascular resistance during exercise. No significant difference was found for the other case history fact ors

Forced expiratory volume during one second ($FEV_{1.0}$) at rest

The mean value for the 20 patients for whom the lowest $FEV_{1.0}$ values, as a percentage of the predicted value were recorded (note that each patient is represented by the highest value that he or she was able to produce) was 50 7 % (range 30 8—63.3) The 20 patients with the highest values had a mean value of 90 2 % (range 77.3—116.3) of the predicted value

The difference between the two groups of patients was significant (*) for age, the estimated total number of attack hours and the estimated mean annual number of sickness points dur ing the period 1957—61 Those with a higher age, those with a higher num ber of sickness points during 1957—61 and those with a greater number of attack hours showed on an average a relatively low forced expiratory vo lume during one second. No significant difference was found for the other case history factors.

Ratio of residual volume to total lung capacity

The mean value for the 20 patients with the highest RV/TLC ratio as a percentage of the predicted value, was 170 9 % (range 153 9—200 0) The patients who were recorded as having the 20 lowest values had a mean value of 114 8 % (range 88.8—132 6) of the predicted value.

For none of the case history factors studied was any significant difference observed between the two groups. There was a marked tendency how ever for those with a higher RV/TLC ratio to have on an average a higher total number of sickness points, but the difference between the two groups of patients did not reach the signific ance level.

To summarize the results of this study on the influence of these case history factors on variables measuring hemodynamic or respiratory function, it may be said that for several of these variables some factors were found to be of statistically significant importan ce. This was valid for mean pressure in the pulmonary artery during exer cise, arterial oxygen saturation during exercise and during anoxic anoxia, pulmonary vascular resistance during exercise and forced expiratory volume during one second at a resting state. The variables for which no factor was found to be of significance were the mean pressure in the pulmonary artery during anoxic anoxia and the ratio of residual volume to total lung capacity With regard to the former group of va riables, it was common to all that age

had a statistically significant influence. Other case history factors with a similar degree of influence on one or more of the variables were the estimated mean annual number of sickness points during the five years almost immediately preceding the investigation, the estimated total number of attack hours, the number of admissions to hospital on account of bronchial asthma and the age at onset of the disease.

Study of the relationship between pairs of variables measuring hemodynamic function, ventilation capacity and lung volumes

A study was made of the relationship between different pairs of each of the variables mentioned on page 84 constituting a measure of hemodynamic function, ventilation capacity or lung volumes.

The material comprised 50 patients, i. e. those men and women who during the heart catheterization performed exercise at 300 and 200 kpm/min respectively. For each of the seven variables the 20 patients showing the least favourable values were selected. The number of patients among these 20 who were common to any two variables was then noted.

Assuming that no relationships existed, the predicted number of persons in each figure in Table 35 would be 7.1. As the table shows, there was a significant relationship (*) between the mean pressure in the pulmonary artery during relatively light muscular exercise and both the *corresponding* pressure during apoxic anoxia and the pulmonary vascular resistance during

	PA_m m.w.	S_{aO_2} m.w.	R m.w.	PA_m a.a.	S_{aO_2} a.a.	$FEV_{1.0}$	RV/TLC
PA_m m.w.		11	13*	13*	12	6	7
S_{aO_2} m.w.	11		12	11	12	11	9
R m.w.	13	12		11	11	9	10
PA_m a.a.	13*	11	11		13	10	6
S_{aO_2} a.a.	12	12	11	13		8	7
$FEV_{1.0}$	9	11	8	10	9		6
RV/TLC	7	9	10	6	7	8	

Table 35. *For pairs of variables the number of patients out of the 20 showing the least favourable values for each of the variables. The degree of significance is assessed.*

PA_m m.w. = mean pressure in pulmonary artery during light muscular work
PA_m [illegible] = during apoxic anoxia
S_{aO_2} m.w. = oxygen saturation in brachial artery during light muscular work
S_{aO_2} [illegible] = during apoxic anoxia
R m.w. = pulmonary vascular resistance during light muscular work
FEV = forced expiratory volume in one second at rest
RV/TLC = residual volume in relation to total lung capacity at rest

exercise. A similarly significant relat ionship (*) was found between the arterial oxygen saturation and the mean pressure in the pulmonary art ery in both cases during anoxic anox ia With regard to the other pairs of variables the observed number of persons common to both variables in each pair exceeded the predicted num ber There was an exception however as regards the relationship between the ratio RV/TLC and both the mean pres sure in the pulmonary artery during muscular work and during anoxic anoxia and the arterial oxygen satura tion during anoxic anoxia

---

Patients with a chronic disease in addition to bronchial asthma

Of the 69 patients below the age of 60 years, who underwent heart catheterization six (three men and three women) had another chronic disease in addition to bronchial asthma, which it was considered might have had a decisive influence on the cardio-respiratory functions. These patients who were not included in the previous analyses of the results obtained during catheterization are reported in Table 38

For th se six patients the experimental conditions were the same as for other

	No	Heart rate beats/min	O saturation per cent Br A	PA	AVD ml/l	O uptake ml/min STPD	Cardiac output l/min	Stroke volume ml	PA M	PCV M	Br A M	R
Rest	6											
mean		94	93	67	47	230	5.5	59	15	7	105	1.6
highest		102	95	73	66	281	6.9	74	20	10	125	3.2
low st		85	91	56	38	206	3.7	36	9	4	90	0 6
Anoxic anoxia	6											
mean		102	68	49	41	231	5.5	54	22		107	
highest		111	74	56	52	257	6.4	66	26		135	
lowest		94	60	36	36	1 3	4.8	43	13		82	
Wo k I	6											
mea		126	91	48	78	7	12.5	96	30	12	132	1.8
highest		143	94	76	98	1007	29.8	210	35	15	163	3.6
lowest		112	87	21	29	685	7 7	56	21	6	90	0 7
W rk II	2											
mean		154	91	34	105	1342	13.1	84	32	13	158	1 4
highest		166	92	38	114	1557	16.5	98	33	14	163	1 4
l west		142	91	29	96	1127	9 9	70	28	12	150	1 4

Tabl 38. Ob ervati ns during heart catheteriz ti n in 6 patients (3 men and 3 women) wh suffered from a hronic disease be ides the bronchial asthma.

Work I=300 kpm/mi for males, 200 kpm/min f females. Work II=600 kpm/min f r males, 400 kpm/min for females. Abbreviations as i T ble 37

patients, with the exception of the fact that one of the men performed 150 kpm/min t the first work load, compared with 300 kpm/min performed by the other men. Since these patient were so small in number the result were nalysed regardless of sex nd age.

On comparison of the mean values for the different function variables (Table 36) with the corresponding fig ures for the other patients (Tables 37—41) considerable differences were found for some of the variables. These differences were not, however stat istically significant. It should be mentioned that none of the patients showed signs of pulmonary hypertension. This group of patients was so small, however and the supervening diseases so varied that no conclusions were drawn.

Groups acc. to sickness points and age (yrs.)		Characteristic	No	Heart rate beats/min	Oxygen saturation per cent		AVD ml/l	Oxygen uptake ml/min STPD
					Br A	PA		
A	30—49	mean	3	64	97 4	74 7	46.4	233
	50—59		6	72	94.6	67.8	48.7	226
	Total A		9	69	95.5	70 1	47.9	233
B	30—49		5	73	95.5	72.8	42.7	216
	50—59		2	75	96.4	69.6	48.9	204
	Total B		7	74	95.8	71.9	44.5	234
C	30—49	"	5	72	98.2	76.6	42.4	226
	50—59		4	87	95.0	68.3	50.1	236
	Total C		9	79	96.8	72.9	45.8	239
Total A+B+C	30—49		13	71	97 0	74 7	43.4	210
"	50—59		12	78	95.0	68.3	48.2	232
Total A+B+C	30—59	mean	25	74	96.0	71 6	46.2	236
		highest		92	99 7	78.6	59 7	293
"	"	lowest		56	87.0	60.6	37.0	178
		SD		11	31.2	5.1	5.9	33
		SE		2.3	0.6	1 1	1.2	6.5

Table 3 *Observations during heart catheterization at rest in male patients*
No =25 except for PCV and R No =23

Group A <1000 sickness points
B 1000—1999
C ≧2000
Br A =brachial artery
PA =pulmonary artery

PCV =pulmonary capillary venous pressure
RA =right atrium
S = systolic pressure
D =diastolic pressure

Groups acc. to sickness points and age (yrs.)		Characteristic	No	Heart rate, beats/min	Oxygen saturation per cent		AVD ml/l	Oxygen uptake ml/min STPD
					Br A	PA		
A	30—49	mean	7	78	96.5	72.2	42.9	203
	50—59		4	72	97.5	70 5	43.0	166
	Total A		11	6	96.0	71 6	42.7	197
B	30—49		9	80	96.2	72.8	42.3	219
	50—59		7	79	98 0	68.3	51 4	213
	Total B		16	80	97.0	70 8	46.3	211
C	30—49		6	79	96.7	72.1	42.2	215
	50—59		5	85	93.4	69.8	43.3	181
	Total C		11	82	96.1	71 1	42.7	200
Total A+B+C	30—49		22	79	96.4	72.4	42.5	209
	50—59		16	79	97 1	69.3	47.3	196
Total A+B+C	30—59	mean	38	79	96.7	1 1	44.5	204
		highest		100	100.0	81 4	57.9	267
"		lowest		59	91 7	63.2	29.7	122
		SD		12	2.3	4.5	6.8	29
		SE		1.9	0 4	0.7	1 1	4 7

Table 38 *Observations during heart catheterization at rest in female patients.*
No =38 except for PVC and R No. =37 Abbreviations as in Table 37

Cardiac output l/min	Stroke volume ml	Pressures, mm Hg RA				PCV				Pulm. vasc. resist. (R)	Pulm. vasc. resist. index (R_s)
		RA	PA			PCV	B A				
		M	S	D	M	M	S	D	M		
5.8	89	1.0	17	7	11	5	130	78	98	1.32	2.44
4.8	87	—0.5	22	8	13	6	151	83	113	1.81	3.53
5.1	74	0	20	8	12	6	144	81	106	1.06	3.06
5.8	81	1.4	17	8	11	5	140	80	106	1.21	2.30
4.2	87	2.5	19	9	12	4	170	90	120	1.94	3.37
5.2	74	1.7	18	8	11	5	145	82	109	1.43	2.56
5.4	75	—1.2	18	5	10	3	132	71	91	1.39	2.41
5.2	80	—0.5	23	7	14	4	145	81	106	1.83	3.40
5.5	85	—0.9	19	6	12	3	138	75	101	1.67	2.83
5.6	81	0.2	17	6	11	4	133	76	100	1.37	2.33
4.8	85	0	23	8	13	5	161	83	112	1.68	3.42
5.2	72	0.2	19	7	12	5	142	79	108	1.49	2.81
7.6	124	5	31	16	20	9	170	110	140	2.77	4.16
3.4	45	—2	12	3	7	1	130	60	80	0.09	0.81
1.1	17	1.9	5	2.9	2	2.4	17.2	13.1	17	0.72	1.28
0.2	2.4	0.4	0.9	0.6	0.7	0.5	3.6	2.7	2.5	0.13	0.37

M = mean pressure
AVD = arterio-venous oxygen difference

$$R = \frac{P_{PA} - P_{PCV}}{Q}$$

$$R_s = \frac{P_{PA} - P_{PCV}}{Q} \text{ BSA}$$

Cardiac output l/min	Stroke volume ml	Pressures, mm Hg RA				PCV				Pulm. vasc. resist. (R)	Pulm. vasc. resist. index (R_s)
		RA	PA			PCV	Br A				
		M	S	D	M	M	S	D	M		
4.5	63	0.7	17	7	11	7	137	79	96	0.85	1.60
4.3	60	0.8	21	7	11	4	148	81	106	1.73	2.78
4.6	62	0.6	18	7	11	6	140	80	100	1.53	2.03
4.1	65	0.9	18	8	12	6	144	80	108	1.35	2.26
4.2	51	0.8	20	9	13	7	171	67	121	1.24	2.10
4.7	80	0.9	19	8	13	6	158	83	114	1.31	2.30
3.3	67	0.3	20	8	13	4	133	73	97	1.39	2.50
4.2	51	0.2	30	8	12	4	149	80	112	1.98	3.20
4.8	66	0.2	30	8	12	4	140	76	104	1.73	2.82
4.1	65	0.7	19	8	12	8	130	78	101	1.27	2.13
4.2	65	0.3	30	9	12	5	158	80	114	1.62	3.65
4.7	81	0.6	19	8	12	9	117	80	107	1.41	2.22
8.0	100	4	29	14	20	11	203	100	145	2.82	4.31
2.9	30	—1	11	3	5	1	110	53	65	0.28	0.33
18.8	11	1.3	3	2.7	4	2.6	31.2	12.8	16	0.74	1.16
1.8	2.2	0.2	0.7	0.4	0.6	0.4	4.1	2.1	3.0	0.12	0.19

Groups acc. to sickness points and age (yrs.)		Characteristic	No	Heart rate beats/min	Oxygen saturation per cent		AVD ml/l	Oxygen uptake ml/min STPD
					Br A	PA		
A	30—49	mean	3	64	97 4	74.7	46 4	233
	50—59	"	6	72	91.6	67.8	48.7	226
	Total A	"	9	69	93.5	70 1	47.9	235
B	30—49		5	73	95.5	72.8	42.7	216
	50—59	"	2	75	96.4	69.6	48.9	204
	Total B	"	7	74	95.8	71 0	44.5	234
C	30—49	"	5	72	98.2	76.6	42.4	226
	50—59		4	87	95.0	68.3	50 1	256
	Total C	"	9	79	96.8	72.9	45.8	239
Total A+B+C	30—49		13	71	97.0	74 7	43.4	210
	50—59		12	78	95.0	68.3	49.2	232
Total A+B+C	30—59	mean	25	4	96.0	71.6	46.2	236
		highest		92	99 7	78.6	50 7	293
		lowest		56	87.0	60.6	37.0	178
	"	SD		11	31.2	5 1	3.9	33
		SE		2.3	0.6	1 1	1.2	6.6

Table 37 *Observations during heart catheterization at rest in male patients*
No =25 except for PCV and R No =23

Group A <1000 sickness points
B 1000—1999 "
C ≧2000
Br A =brachial artery
PA =pulmonary artery

PCV =pulmonary capillary venous pressure
RA =right atrium
S =systolic pressure
D =diastolic pressure

Groups acc. to sickness points and age (yrs.)		Characteristic	No	Heart rate, beats/min	Oxygen saturation per cent		AVD ml/l	Oxygen uptake ml/min STPD
					Br A	PA		
A	30—49	mean	7	78	96.5	72.2	42.9	203
	50—59		4	72	97.5	70.6	43.0	186
	Total A		11	76	96.9	71 6	43 7	197
B	30—49		9	80	96.2	72.8	42.3	210
	50—59		7	79	98.0	68.3	51 4	213
	Total B		16	80	97.0	70.6	46.3	211
C	30—49		6	79	96.7	72 1	42.2	216
	50—59		5	85	95 4	69 8	43 3	181
	Total C		11	82	96.1	71 1	42.7	200
Total A+B+C	30—49		22	79	96.4	72.4	42.5	209
	50—59		16	79	97 1	69.3	47.3	196
Total A+B+C	30—59	mean	38	79	96.7	71 1	44.5	204
		highest		100	100 0	81 4	37 9	207
		lowest		59	91 7	63.2	29.7	122
		SD		12	2.3	4.5	6.8	29
		SE		1 9	0 4	0.7	1 1	4 7

Table 38. *Observations during heart catheterization at rest in female patients.*
No =38, except for PVC and R No. =37 Abbreviations in Table 37

Oxygen uptake ml/min STPD	Cardiac output l/min	Stroke volume ml	Pressures, mm Hg					
			PA			Br A		
			S	D	M	S	D	M
229	5.4	76	24	9	15	120	65	87
218	5.5	68	33	14	20	148	78	101
222	5.5	71	30	12	18	130	74	96
228	6.4	73	28	12	18	120	63	84
186	4.6	63	27	13	18	161	82	106
216	5.9	66	28	17	18	179	88	90
218	6.5	66	24	9	15	119	62	82
220	4.7	45	49	19	30	147	75	101
223	5.1	59	35	13	23	131	66	92
221	5.8	73	26	10	16	130	63	84
216	5.1	63	37	16	22	148	78	104
230	4.5	65	31	12	19	123	70	94
281	3.6	100	73	32	47	179	90	120
165	3.6	41	13	6	9	85	35	50
33	1.3	16	14	7.9	10	27.3	15.6	19
6.4	0.8	3.2	2.1	1.6	3.0	5.8	3.3	3.9

Oxygen uptake ml/min STPD	Cardiac output l/min	Stroke volume ml	Pressures, mm Hg					
			PA			B A		
			S	D	M	S	D	M
180	4.3	61	25	10	16	117	65	88
167	4.3	60	31	11	18	140	81	101
17[illegible]	4.2	61	27	10	17	125	71	94
181	4.7	56	28	11	17	131	71	96
188	4.1	49	28	12	18	158	80	108
189	4.6	63	26	11	17	142	75	101
182	4.6	52	28	11	18	126	68	92
163	3.9	44	31	12	16	135	69	97
173	4.3	48	29	11	17	130	69	94
18[illegible]	4.5	63	26	11	17	125	68	92
174	4.1	47	29	12	17	145	77	104
180	4.2	31	27	11	17	133	72	97
267	7.1	77	43	28	32	190	108	145
132	2.7	31	16	6	8	82	50	58
30	10.3	11	7	4.6	5	21.9	12.3	17
4.9	1.7	1.8	1.1	0.7	0.8	4.0	2.0	2.8

Groups acc. to sickness points and age (yrs.)		Characteristic	No	Heart rate beats/min	Oxygen saturation per cent		AVD ml/l
					Br A	PA	
A	30—49	mean	3	72	78.0	56.5	42.8
	50—59		6	82	76.0	50.7	40.7
	Total A		9	79	76.0	52.8	41.3
B	30—49	"	5	86	71.4	54.5	34.2
	50—59		2	89	75.0	52.3	41.2
	Total B	"	7	87	4.6	55.9	38.2
C	30—49		5	81	82.7	61.4	40.9
	50—59	"	4	100	70.2	43.8	49.5
	Total C		9	89	77.2	53.6	41
Total A+B+C	30—49	"	13	81	78.4	57.6	35.7
	50—59	"	12	80	73.4	48.7	43.7
Total A+B+C	30—59	mean	25	85	6.0	53.2	41.1
		highest		118	85.1	71.9	58.0
"		lowest		60	54.8	33.9	30.8
		SD		14	6.9	8.9	6.7
"	"	SE		2.8	1.8	1.8	1.3

Table 39 Observations during anoxic anoxia on heart catheterisation in male patients
Abbreviations as in Table 37

Groups acc. to sickness points and age (yrs.)		Characteristic	No.	Heart rate beats/min	Oxygen saturation per cent		AVD ml/l
					Br A	PA	
A	30—49	mean	7	80	7_.9	51.5	42.0
	50—59		4	88	78.1	48.7	40.1
	Total A		11	83	74.9	50.6	41.3
B	30—49		9	84	80.7	56.1	42.9
	50—59		7	88	75.8	59.0	45.0
	Total B		16	86	78.7	53.4	43.7
C	30—49		6	88	78.2	53.2	41.6
	50—59		5	90	71.3	46.3	41.4
	Total C		11	89	75.1	50.1	41.6
Total A+B+C	30—49		22	85	78.5	53.8	4_.3
	50—59		16	88	73.6	46.5	42.5
Total A+B+C	30—59	mean	38	86	76.5	51.6	43.4
		highest		112	91.5	61.3	64.2
		lowest		55	63.4	28.6	27.7
		SD		13	6.7	7.8	7.6
		SE		2.1	1.4	1.3	1.3

Table 40 Ob[illegible]rvation during anox[illegible] anoxia on heart catheterisati[illegible]n in female patient[illegible]
Abbreviations as in Table 37

Stroke volume ml	Pressures, mm Hg										Pulm. vasc. resist. (R)	Pulm. vasc. resist. index (R_I)
	PA			PCV		Br A						
	S	D	M	No.	M	No.	S	D	M	No.		
107	28	11	16	3	4	3	172	83	113	2	1.30	2.34
91	45	29	28	4	10	4	194	97	134	4	1.73	3.38
97	30	17	24	6	8	7	185	91	128	6	1.60	3.03
83	33	15	22	5	7	6	158	79	114	3	1.48	2.66
84	41	30	37	1	13	2	178	93	131	1	1.30	3.34
90	38	10	23	6	8	7	164	83	119	8	1.43	2.61
85	25	8	15	5	6	4	149	78	100	4	0.92	1.79
66	33	22	33	4	7	4	201	98	138	4	3.00	5.37
82	30	15	24	9	6	8	173	87	122	8	2.01	3.58
97	29	12	18	12	6	12	139	79	110	11	1.23	2.29
81	48	21	30	9	9	10	194	97	134	9	2.30	4.15
90	38	16	24	21	7	22	175	87	122	20	1.71	3.13
130	97	42	63		13		230	120	163		6.38	10.66
67	12	3	8		3		130	70	100		0.50	0.78
90	17	9.0	12		3.3		29.1	14.4	21		1.23	2.21
4.0	3.8	1.9	2.4		0.7		6.2	3.1	4.3		0.30	0.60

Stroke volume ml	Pressures, mm Hg										Pulm. vasc. resist. (R)	Pulm. vasc. resist. index (R_I)
	PA			PCV		Br A						
	S	D	M	No.	M	No.	S	D	M	No.		
102	28	11	17	2	4	3	183	93	128	2	1.83	1.84
107	47	21	35	4	10	4	218	103	151	3	1.57	3.90
103	40	17	27	6	8	7	208	98	141	5	1.25	2.39
91	38	13	23	4	8	5	181	86	118	4	1.20	1.66
72	43	25	35	0		1	225	105	150	0		
88	39	17	25	4	8	6	185	89	133	4	1.30	1.86
83	30	10	17	4	6	4	184	85	113	3	0.94	1.70
86	43	11	23	0		1	180	75	110	0		
88	33	10	18	4	6	5	167	83	112	3	0.91	1.70
96	35	12	19	10	6	12	179	87	119	9	1.08	1 [illegible]
96	48	30	33	4	10	4	213	98	144	3	1.57	3.09
96	35	15	23	14	7	18	100	91	127	12	1.30	3.03
147	74	35	62		13		230	118	170		2.14	4.56
44	17	4	6		3		130	70	100		0.16	0.30
23	14	7.8	11		3.6		23.6	15.8	21		[illegible]	1.12
4.3	2.1	1.7	2.8		1.0		7.9	3.7	5.7		0.17	0.32

Groups acc. to sickness points and age (yrs.)		Characteristic	No.	Heart rate, beats/min	Oxygen saturation per cent		AVD ml/l	Oxygen uptake ml/min STPD	Cardiac output l/min
					Br A	PA			
A	30—49	mean	3	93	96.4	51.0	92.7	850	9.7
	50—59	„	5	100	93.2	41.9	96.5	867	9.1
	Total A	„	8	107	94.4	45.3	95.1	872	9.3
B	30—49		5	108	94.9	47.4	88.7	885	10.0
	50—59		2	91	91.8	44.7	94.8	869	9.2
	Total B		7	103	94.0	46.6	90.4	880	9.8
C	30—49		5	98	98.7	56.1	82.6	740	9.0
	50—59	„	4	124	91.7	37.3	104.4	854	8.1
	Total C		9	110	95.6	47.7	92.3	791	8.6
Total A+B+C	30—49	„	13	101	96.7	51.0	87.3	826	9.5
„	50—59	„	11	107	93.0	40.7	99.1	863	8.8
Total A+B+C	30—50	mean	24	104	95.0	46.6	92.7	844	9.2
		highest		140	99.9	60.7	111.2	1016	12.1
„		lowest		78	82.6	27.8	71.0	510	6.6
„	„	SD		14	4.2	8.6	10.5	108	1.4
„		SE		2.8	0.0	1.8	2.2	22.1	0.3

Table 41. *Observations on heart catheterization during work 300 kpm/min in male patients.* Abbreviations as in Table 37

Groups acc. to sickness points and age (yrs.)		Characteristic	No.	Heart rate, beats/min	Oxygen saturation per cent		AVD ml/l	Oxygen uptake ml/min STPD	Cardiac output l/min
					Br A	PA			
A	30—49	mean	3	129	96.2	42.0	113.6	1344	12.2
	50—59		5	120	90.3	33.9	101.8	1393	13.7
	Total A		8	120	92.5	36.9	108.1	1375	13.1
B	30—49		5	136	92.9	36.6	107.3	1308	12.8
	50—59		1	158	90.7	27.5	132.9	1496	11.3
	Total B		6	140	93.5	35.1	111.6	1389	12.6
C	30—49		5	130	98.8	44.1	110.0	1383	1_.9
	50—59		1	153	97.5	32.3	128.1	1297	10.1
	Total C		6	134	98.6	42.1	113.0	1366	12.3
Total A+B+C	30—49		13	132	95.9	40.7	109.8	1367	12.7
	50—59		7	137	92._	32.8	112.1	1394	12.8
Total A+B+C	30—50	mean	20	134	94.8	37.9	110.0	1377	12.7
		highest		166	100.0	40.7	133.4	1305	19.6
		lowest		100	74.3	24.0	74.4	1069	8.9
		SD		17	0.3	7.2	20.0	114	2.4
		SE		3.7	1.4	1.6	4.4	4.4	0.5

Table 42. *Observations on heart catheterization during work 600 kpm/min, in male patients.* Abbreviations as in Table 37

Stroke volume ml	Pressures, mm Hg PA S	D	M	PCV No.	M	B A No.	S	D	M	No.	Pulm. vasc. resist. (R)	Pulm. vasc. resist. index (R_I)
73	36	11	18	5	6	6	163	85	118	5	1.84	2.51
61	42	17	26	4	8	3	187	90	135	4	2.21	3.63
64	34	13	21	9	7	9	171	86	121	9	1.85	3.01
73	30	14	20	6	9	6	165	88	126	5	1.21	2.14
76	32	13	20	4	9	3	207	98	143	4	1.29	2.26
72	31	14	20	9	9	11	186	93	124	9	1.30	2.19
78	35	13	21	5	9	5	167	82	115	4	1.80	2.84
61	37	14	23	2	9	4	200	93	130	2	1.87	2.77
72	36	16	22	7	9	9	182	87	126	6	1.82	2.82
73	31	13	20	15	8	17	166	88	120	14	1.63	2.47
6[illegible]	36	14	23	10	9	12	200	94	140	10	1.70	2.91
71	33	14	21	23	8	29	168	89	128	21	1.84	2.63
100	48	27	33		15		245	110	170		3.29	4.94
43	20	8	10		4		120	65	93		0.80	0.88
13	8	4.3	6		2.8		25.0	12.8	22		0.75	1.16
2.3	1.4	0.9	1.1		0.5		6.8	2.4	4.1		0.15	0.23

Stroke volume ml	Pressures, mm Hg PA S	D	M	PCV No.	M	Br A No.	S	D	M	No.	Pulm. esc. resist. (R)	Pulm. vasc. resist. index (R_I)
77	36	13	21	8	9	7	178	91	122	6	1.26	2.07
70	45	13	24	2	13	3	205	91	128	2	1.78	2.93
73	39	14	22	8	10	10	186	92	127	8	1.30	2.29
77	33	13	22	7	9	7	195	94	130	7	1.17	1.94
70	34	11	18	4	9	4	208	98	140	3	1.33	2.13
7[illegible]	33	14	21	11	9	11	200	93	124	10	1.19	2.00
74	33	18	21	4	9	4	178	85	120	4	0.82	1.31
65	34	13	23	1	10	2	185	85	123	1	1.87	2.79
71	35	13	22	5	9	7	181	88	124	5	1.03	1.61
[illegible]	33	14	21	17	9	19	183	91	124	17	1.12	1.84
80	38	13	22	7	10	9	202	96	135	6	1.53	2.51
74	36	14	21	24	9	25	190	93	125	23	1.23	2.02
10[illegible]	53	23	31		15		270	120	180		2.18	3.60
62	20	7	14		1		122	70	82		0.50	0.72
13	8	4.3	6		2.8		22.3	12.7	21		0.50	0.63
2.4	1.4	3.4	1.1		0.6		8.1	2.4	3.6		1.05	0.17

7 Irnell

Groups acc. to sickness points and age (yrs.)		Characteristic	No	Heart rate beats/min	Oxygen saturation per cent		AVD ml/l	Oxygen uptake ml/min STPD	Cardiac output l/min
					Br A	PA			
A	30—49	mean	6	108	96.8	48.6	86.5	686	8.0
	50—59	"	4	114	96.0	39.5	97.4	670	6.9
	Total A		10	110	96.7	45.0	90.9	679	7.6
B	30—49	"	6	107	96.0	47.0	88.4	660	7.6
	50—59	"	6	115	94.4	42.9	90.2	740	8.0
	Total B		12	110	95.2	45.1	89.3	703	7.8
C	30—49	"	6	116	97.0	50.1	82.0	721	8.9
	50—59	"	4	123	94.8	42.7	90.4	712	7.9
	Total C		10	118	96.7	47.1	85.4	717	8.5
Total A+B+C	30—49	"	18	110	96.9	48.6	85.6	692	8.2
	50—59		14	117	95.1	41.8	92.5	712	7.6
Total A+B+C	30—59	mean	32	113	96.1	45.7	88.5	701	8.0
"	"	highest		141	99.3	55.6	104.2	964	11.9
"		lowest		81	91.2	31.6	69.5	522	6.2
		SD		14	5.0	5.7	8.9	101	12.6
		SE		2.5	0.9	1.0	1.6	17.8	2.2

Table 43. *Observations on heart catheterization during work, 200 kpm/min, in female patients. Abbreviations as in Table 37*

Groups acc. to sickness points and age (yrs.)		Characteristic	No	Heart rate beats/min	Oxygen saturation per cent		AVD ml/l	Oxygen uptake ml/min STPD	Cardiac output l/min
					Br A	PA			
A	30—49	mean	7	122	96.4	41.0	99.2	1071	10.8
	50—59		3	142	97.3	35.1	108.4	929	8.5
	Total A		10	136	96.7	39.0	102.0	1028	10.1
B	30—49		7	135	97.1	41.1	101.7	1010	10.0
	50—59		5	141	96.9	34.7	102.9	1058	9.6
	Total B		12	138	97.0	38.6	102.1	1030	9.9
C	30—49		4	140	96.9	40.0	93.8	10[illegible]0	10.9
	50—59		2	169	93.2	33.4	109.1	1111	10.2
	Total C		6	152	95.7	37.8	102.2	1076	10.7
Total A+B+C	30—49		18	141	96.8	41.2	100.2	1045	10.5
	50—59		10	139	96.2	34.6	106.1	1027	9.3
Total A+B+C	30—59	mean	28	140	96.6	38.9	102.1	1039	10.1
		highest		171	98.7	47.1	116.9	1416	13.1
		lowest		100	92.4	23.8	79.6	752	6.1
		SD		16	1.4	5.0	10.6	131	12.6
		SE		3.0	0.3	1.1	2.0	23.3	2.4

Table 44. *Observations on heart catheterization during work, 400 kpm/min, in female patients.* Abbreviations as in Table 37

the impression of the author that the patients in the asthma series adopted a particularly positive attitude to the investigations and attempted to show that it was in their own interest to produce the maximum achievement possible.

For this series of patients regarded as a whole the *lung volumes* vital capacity and total lung capacity were on an average significantly smaller than predicted. In this respect, there was no sex difference of any notable order of size. The relatively low total lung capacity may have some connec tion with the reduction in vital capac ity which was an average finding The reduction in vital capacity may be explained as partly due to hyperinfla tion, as suggested by the relatively large residual volume this may be connected with the fact that these patients — regarded as a group — seemed to be less physically active than is usual

Several authors have emphasized the poor correlation between vital capacity and pulmonary disease. It is not uncommon, especially in patients with chronic obstructive pulmonary diseases, to find a relatively high value for the ital capacity while at the same time the ventilation capacity is found to be markedly reduced (Baldwin, Cournand and Dickinson, 1949) Different authors have obtained different ideas of the order of size of the vital capacity in patients with chronic obstructive pulmonary disease. Hurtado, Kaltreider Fray Brooks and McCann (1934) Beale Fowler and Comroe (195) and Park and Lee (1961) generally found reduced values in these patients, while Herschfus, Bresnick and Segal (1953) found a mean value somewhat exceeding the predicted value A significantly higher value for both the ratios FRC/TLC and RV/TLC was found on an average in both sexes in the present asthma series on comparison with the control material On classification of the asthma series into groups according to the over all degree of severity of the disease it was found that on an average the female groups with a high total number of sickness points attained significantly higher values than the group with a low number This applied both to FRC/TLC and RV/TLC. Some difference was also found for the men, but this was not significant. On assessing these ratios consideration should be taken of the fact that the total lung capacity value for the material as a whole — and also when classified into groups according to sickness points — was lower than the predicted value The results obtained in these tests thus indicate that there was a certain degree of relationship, as expected, between the over-all degree of severity of the disease and the size of the ratio of both the residual volume and the functional residual capacity to the total lung capacity

The relationship between asthma and pulmonary emphysema has long been discussed. According to certain clinical observations asthma often leads to the development of pulmonary emphysema (Abbott, Hopkins, van Flait and Robinson, 1953 *inter alia*). Some histological investigations of patients with asthma (Gough, 1952 Williams and Leopold, 1959 among

Discussion

The degree of severity of the bronchial asthma in this series of patients varied greatly and it was expected that any secondary effects on the cardiac and pulmonary functions might also vary. The patients were therefore classified into groups according to several different principles meant to represent the degree of severity of the disease as found in the case histories. The investigation findings were then compared between these groups. A further aim was to compare the asthma series — or groups of the series — with suitable control material. In the comparisons concerning ventilation capacity and lung volumes the control material used was that reported by Berglund, Birath, Bjure, Grimby, Kjellmer, Sandqvist and Soderholm (1963), Birath, Kjellmer and Sandqvist (1963) and Grimby and Söderholm (1963). The comparisons with regard to physical work capacity were made with a control material comprising individuals from the City of Uppsala Health Survey of 1961 (Irnell and Linder, to be published). The hemodynamic investigation findings from the asthma material were compared in several cases with the results of a study by Holmgren, Jonsson and Sjöstrand (1960).

For comparisons with regard to respiratory function the choice of the control material lay primarily between that mentioned above and the series studied by Kory, Callahan, Borén and Syner (1961). The deciding factors for the choice of the former were as follows:

1. The investigations were based on Swedish persons.
2. In principle the same apparatus and same mode of procedure were used as in the present study.
3. The study treated both ventilation capacity and lung volumes.
4. There was a wide age range among the group of persons studied.
5. The material appeared to be relatively representative of the general population.

Of especial importance was the fact that the apparatus and mode of procedure were in principle the same in the present study of the asthma series and the study of the control subjects.

Apart from these factors, however, the degree of motivation in both the subject and the investigator appears to be of importance. In assessing the extent to which normal subjects have co-operated, a comparison between two independent function studies may be of value. In this control the high degree of correlation between the two tests FEV$_{1.0}$ and MVV$_F$ may be utilized. This relationship should however depend on the condition that the respiration is not impaired by variable air way obstruction. The strain on the subject, especially in the test of the maximum ventilation capacity, leads in several patients with bronchial asthma to the provocation of some degree of respiratory tract obstruction, even when the test is performed at an optimal period with respect to the asthma. No definite objective measure of the degree of motivation could be obtained, but it is

according to the estimated degree of severity of the disease. These results thus support the view that there was some degree of relationship, as expected, between the estimated degree of severity and the ventilation capacity value found.

The degree of deviation between the observed and predicted values should be regarded with some caution, however since there is no available control material that simultaneously fulfils all the criteria of "normal" material in the true sense of this word, which include the demand for a completely random selection of subjects from the total population.

It is probable that the differences found between the asthma series and the control material can be explained to a certain extent by the fact that the methods were not identical in practical use although identical in principle. The differences between the mean values for the two series were, however of a considerable order of size, and it was also found that with regard to the variables for which the difference was greatest there was also a considerable difference between group A and group C, i. e. between the group that was estimated by the number of sickness points to have the greatest degree of severity since the *onset of* the asthma, and the group with the lowest degree.

On studying the mean deviation from the predicted value for each of the test $FEV_{1.0}$, $FEV_{\%}$, MVV_{40} and MVV_{f}, for the whole main asthma series, it was found that the deviation for the latter two was the greatest, i. e. about 40 percent units. The corresponding deviations for $FEV_{1.0}$ and $FEV_{\%}$ were about 30 and 20 percent units respectively. This supports the view that a test of maximum ventilation capacity is to be preferred for an assessment of the reduction in ventilatory function in a material such as this. The possible advantage to the subject in being able to choose his own respiratory frequency did not appear to be greater for the patients in the asthma series than for the control persons. Regardless of whether a fixed frequency of 40 breaths per minute was used in the maximum ventilation capacity test or whether the patient himself chose the frequency the asthma patients on an average attained practically the same percentage of the predicted value. Thus for the asthma patients one of these two tests was probably adequate for assessing the influence of the disease on the ventilatory function. This assumption is supported by the fact that when a selection was made from the 110 asthma patients of firstly the 20 patients with the least favourable values at the MVV_{40} test and secondly the 20 patients with the least *favourable values* at the MVV_{f} test, no *fewer than 1*[illegible] were common to each group. This indicated a highly significant relationship between these two tests of ventilation capacity.

The somewhat smaller mean difference from the control material shown by the $FEV_{1.0}$ test, compared with the MVV tests, is probably connected, *inter alia* with the smaller degree of physical exertion associated with the performance of this test. The even smaller difference with regard to $FEV_{\%}$ is due

others) however have contradicted the view that the incidence of emphysema is higher in patients with asthma Gloor (1954) arrived at the opposite conclusion This contradiction may be explained in the words of Kelp (1961) "Wir glauben dass die Diskrepanz zwischen klinischen und histopathologischen Ergebnissen zum Teil auf einer unterschiedlichen Auslegung des Emphysembegriffes beruht Viele Kliniker bezeichnen jede Lungenüberblähung zumal wenn sie längere Zeit besteht als Emphysem während von pathologisch anatomischer Seite das Vordhandensein bestimmter morphologischer Merkmale für die Diagnose eines Emphysems im engeren Sinne gefordert wird"

An increased ratio of FRC/TLC and RV/TLC will be subsequently referred to in this discussion as 'hyperinflation' and will be regarded as being consistent with but not specifically evidential of emphysema

In comparison with the control material the asthma series as a whole showed on an average a significantly reduced *ventilation capacity* Large variations were found in this series for each of the different tests. These variations however were observed not only for the material as a whole but also within the groups formed according to factors representing the estimated degree of severity of the disease, such as the number of sickness points the duration etc. One possible reason is that the patients — in spite of the fact that in reality their asthma was of similar degrees of severity — exhibited considerable differences in ventilation capacity This could be due to individual dissimilarities for example the difference in the capacity of the lung tissue to resist strain of the type caused by bronchial constriction. The different errors of the method also play a role The error in estimating the degree of severity of the disease, for example, is an important factor in the analysis of the results The fact that an error of method exists is due not only to lack of memory and expressive ability on the part of some patients, but also to the fact that different patients do not measure the degree of severity in the same way and experience their symptoms differently even when these are in principle of the same type and grade Another factor of considerable importance was the difficulty in ascertaining that the subjective condition of a patient at the time of an investigation was optimal with respect to the asthma. These factors, together with others such as motivation muscular strength, degree of training etc., contribute to the relatively wide ranges in the ventilation capacity and lung volumes even in groups of patients in whom the asthma was assessed as having been of a relatively similar degree of severity This line of reasoning obviously holds in principle for the physical work capacity and hemodynamic functions also

When the mean observed values for $FEV_{1.0}$, $FEV_{\%}$, MVV_{40} and MVV_{f} were calculated separately for the asthma material as a whole a highly significant difference was found from the mean predicted values. For each of these tests significant differences were also shown between the different groups on classification of the material

according to the estimated degree of severity of the disease These results thus support the view that there was some degree of relationship as expected, between the estimated degree of severity and the ventilation capacity value found.

The degree of deviation between the observed and predicted values should be regarded with some caution, however since there is no available control material that simultaneously fulfils all the criteria of "normal material in the true sense of this word, which include the demand for a completely random selection of subjects from the total population.

It is probable that the differences found between the asthma series and the control material can be explained to a certain extent by the fact that the methods were not identical in practical use although identical in principle The differences between the mean values for the two series were however of a considerable order of size, and it was also found that with regard to the variables for which the difference was greatest there was also a considerable difference between group A and group C, i. e. between the group that was estimated by the number of sickness points to have the greatest degree of severity since the onset of the asthma, and the group with the lowest degree

On studying the mean deviation from the predicted value for each of the test $FEV_{1.0}$, FEV_x, MVV_{40} and MVV_f for the whole main asthma series, it was found that the deviation for the latter two was the greatest, i. e. about 40 percent units. The corresponding deviations for $FEV_{1.0}$ and FEV_x were about 30 and 20 percent units respectively This supports the view that a test of maximum ventilation capacity is to be preferred for an assessment of the reduction in ventilatory function in a material such as this The possible advantage to the subject in being able to choose his own respiratory frequency did not appear to be greater for the patients in the asthma series than for the control persons. Regardless of whether a fixed frequency of 40 breaths per minute was used in the maximum ventilation capacity test or whether the patient himself chose the frequency the asthma patients on an average attained practically the same percentage of the predicted value. Thus for the asthma patients one of these two tests was probably adequate for assessing the influence of the disease on the ventilatory function. This assumption is supported by the fact that when a selection was made from the 116 asthma patients of firstly the 20 patients with the least favourable values at the MVV_{40} test, and secondly the 20 patients with the least favourable values at the MVV_f test no fewer than 17 were common to each group This indicated a highly significant relationship between these two tests of ventilation capacity

The somewhat smaller mean difference from the control material shown by the $FEV_{1.0}$ test, compared with the MVV tests, is probably connected, *inter alia* with the smaller degree of physical exertion associated with the performance of this test. The even smaller difference with regard to FEV_x is due

others) however have contradicted the view that the incidence of emphysema is higher in patients with asthma. Gloor (1954) arrived at the opposite conclusion This contradiction may be explained in the words of Kelp (1961) "Wir glauben, dass die Diskrepanz zwischen klinischen und histopathologischen Ergebnissen zum Teil auf einer unterschiedlichen Auslegung des Emphysembegriffes beruht. Viele Kliniker bezeichnen jede Lungenüberblähung zumal wenn sie längere Zeit besteht, als Emphysem während von pathologisch anatomischer Seite das Vordhandensein bestimmter morphologischer Merkmale für die Diagnose eines Emphysems im engeren Sinne gefordert wird"

An increased ratio of FRC/TLC and RV/TLC will be subsequently referred to in this discussion as "hyperinflation and will be regarded as being consistent with but not specifically evidential of emphysema

In comparison with the control material the asthma series as a whole showed on an average a significantly reduced *ventilation capacity* Large variations were found in this series for each of the different tests These variations, however were observed not only for the material as a whole but also within the groups formed according to factors representing the estimated degree of severity of the disease, such as the number of sickness points, the duration etc One possible reason is that the patients — in spite of the fact that in reality their asthma was of similar degrees of severity — exhibited considerable differences in ventilation capacity This could be due to individual dissimilarities, for example the difference in the capacity of the lung tissue to resist strain of the type caused by bronchial constriction. The different errors of the method also play a role The error in estimating the degree of severity of the disease, for example, is an important factor in the analysis of the results The fact that an error of method exists is due not only to lack of memory and expressive ability on the part of some patients but also to the fact that different patients do not measure the degree of severity in the same way and experience their symptoms differently even when these are in principle of the same type and grade. Another factor of considerable importance was the difficulty in ascertaining that the subjective condition of a patient at the time of an investigation was optimal with respect to the asthma. These factors, together with others such as motivation, muscular strength degree of training etc., contribute to the relatively wide ranges in the ventilation capacity and lung volumes even in groups of patients in whom the asthma was assessed as having been of a relatively similar degree of severity This line of reasoning obviously holds in principle for the physical work capacity and hemodynamic functions also.

When the mean observed values for $FEV_{1.0}$, $FEV_{\%}$, MVV_{40} and MVV_{f} were calculated separately for the asthma material as a whole a highly significant difference was found from the mean predicted values For each of these tests significant differences were also shown between the different groups on classification of the material

according to the estimated degree of severity of the disease. These results thus support the view that there was some degree of relationship, as expected, between the estimated degree of severity and the ventilation capacity value found.

The degree of deviation between the observed and predicted values should be regarded with some caution, however since there is no available control material that simultaneously fulfils all the criteria of "normal" material in the true sense of this word, which include the demand for a completely random selection of subjects from the total population.

It is probable that the differences found between the asthma series and the control material can be explained to a certain extent by the fact that the methods were not identical in practical use although identical in principle. The differences between the mean values for the two series were, however, of a considerable order of size, and it was also found that with regard to the variables for which the difference was greatest there was also a considerable difference between group A and group C, i.e. between the group that was estimated by the number of sickness points to have the greatest degree of severity since the onset of th[illegible] sthma and the group with the lowest degree.

On [illegible]tudying the mean deviation from the predicted value for each of the test $FEV_{1.0}$, FEV_{x}, MVV_{40} and MVV_{f}, for the whole main asthma series it was found that the deviation for the latter two was the greatest, i.e. about 40 percent units. The corresponding deviations for $FEV_{1.0}$ and FEV_{x} were about 30 and 20 percent units respectively. This supports the view that a test of maximum ventilation capacity is to be preferred for an assessment of the reduction in ventilatory function in a material such as this. The possible advantage to the subject in being able to choose his own respiratory frequency did not appear to be greater for the patients in the asthma series than for the control persons. Regardless of whether a fixed frequency of 40 breaths per minute was used in the maximum ventilation capacity test or whether the patient himself chose the frequency the asthma patients on an average attained practically the same percentage of the predicted value. Thus for the asthma patients one of these two tests was probably adequate for assessing the influence of the disease on the ventilatory function. This assumption is supported by the fact that when a selection was made from the 118 asthma patients of firstly the 20 patients with the least favourable values at the MVV_{40} test, and secondly the 20 patients with the least favourable values at the MVV_{f} test, no fewer than 17 were common to each group. This indicated a highly significant relationship between these two tests of ventilation capacity.

The somewhat smaller mean difference from the control material shown by the $FEV_{1.0}$ test, compared with the MVV tests, is probably connected, inter alia with the smaller degree of physical exertion associated with the performance of this test. The even smaller difference with regard to FEV_{x} is due

to the fact that the mean vital capacity showed some reduction in comparison with the control material

The four indices measuring ventilation capacity agree as regards the differences between the mean value for those patients who did not attain as many as 1000 total sickness points and that for the patients who attained a higher number

A regression analysis of the importance of certain case-history data to the results of different variables measuring respiratory function was performed. By this means a relationship was found between the intensity of the disease during the five-year period almost immediately preceding the investigation (1957—61) and the results of the actual function tests for the variables $FEV_{1.0}$, $FEV_{\%}$, MVV_{40} and MVV_{I}. Five different interpretations may be suggested for this relationship

1 The intensity of the disease had a deteriorating effect on the ventilation capacity but this did not influence the intensity of the disease. With this interpretation the sickness points for 1957—61 constitute a genuine factor (a measure of the intensity) that would explain the ventilation capacity value found at the present study

2 The ventilation capacity had remained relatively stationary during a succession of years and had influenced the number of days of incapacity. In other words the intensity of the disease during 1957—61 was causally dependent upon the ventilation capacity and not vice versa.

3 There was reciprocal effect between the intensity of the disease and the ventilation capacity, for example the intensity of the disease during 1957 influenced the lung function state for the same year and this in turn influenced the intensity of the disease during 1958, and so on.

4 Both the intensity of the disease and the ventilation capacity were influenced in the same direction by other factors, e g certain forms of therapy. In other words both the disease intensity and the ventilation capacity were causally dependent on a common factor

5 Some patients behaved according to point 1, others according to point 2 and still others according to point 3 and so on, or according to combinations of these alternatives

The present study does not permit any conclusions, on the basis of purely statistical data as to which of these interpretations is correct. Some hypothetical reasoning is, however, possible. The relationship between the intensity of the disease during the years previous to 1957 and the ventilation capacity at the present investigation was found on analysis to be negligible. If the relationship between the ventilation capacity at the beginning of the 1950 s and that at the beginning of the 1960 s were such that slow change (improvement or deterioration) of this function had occurred, alternative 1 could hardly be applicable. If it had applied here, the

partial correlation between the ventilation capacity at the present investigation and the intensity of the disease before 1957 — eliminating the disease intensity for the period 1957—61 — would clearly deviate from zero. However the partial correlation between the disease intensity before 1957 and the ventilation capacity at the present investigation — eliminating the influence of the disease intensity of 1957—61—was weak, varying between —0.06 and +0.04 for the variables FEV$_{1.0}$, FEV%, MVV$_{40}$ and MVV$_f$. In order to determine whether the reduction in ventilation capacity is slowly reversible or can change markedly during a period of five years, a follow up study would be required. It is as yet too soon to perform such a study on the patients of the present series.

With regard to alternatives 2 and 3 a follow up study would be desirable from the point of view of obtaining a measure of the relationship between the ventilation capacity at two different time points with an interval of 5—10 years.

On the basis of clinical experience it may be said that a definite impression is obtained that in the short view the ventilation capacity is dependent on the intensity of the disease. As to the long term relationship it does not seem possible to express an opinion with certainty in cases of uncomplicated bronchial asthma.

With regard to alternative 4 there appeared to be no common factors that could have had appreciable influence on the ventilation capacity and the disease intensity simultaneously in the same favourable direction. On the other hand there were factors that probably influenced the intensity of the disease by preventing or counteracting the occurrence of for example, asthmatic attacks (e.g. corticosteroids). Other factors of the type that may influence the ventilation capacity as such probably also played some role (e.g. breathing exercises). If both of these forms of therapy occur at the same time they then acquire the character of a common factor. During the period 1957—61 it was only in exceptional cases among these patients that therapy of both these types was given, and therefore alternative 4 does not appear to be worthy of appreciable consideration.

On regression analysis a relationship was found between the over all degree of severity of the disease and the ratio of the residual volume in particular but also the functional residual capacity to the total lung capacity. This relationship did not appear to depend primarily on the intensity of the disease during the years almost immediately preceding the time of the investigation on analysis the relationship between these ratios and the intensity of the disease during the years mentioned was found to be relatively small. The reversibility factor appeared to be greater with regard to the effect of the disease on the ventilation capacity — as expressed by the function tests FEV$_{1.0}$, FEV%, MVV$_{40}$ and MVV$_f$ — than with regard to the effect on certain lung volume relations — as expressed by the ratios of not only the residual volume but also the functional residual capacity to the total lung capacity.

The *physical work capacity* of the patients in the asthma material was determined in order to obtain a quantitative assessment of the respiratory and circulatory functional impairment *in particular* directly or indirectly caused by the disease.

Work tests as methods of clinical investigation in pulmonary diseases have been used previously among others by Dahlstrom (1955 and 1957) on patients with pulmonary tuberculosis and by Svanborg (1961) on patients with pulmonary sarcoidosis. Both authors found *on an average* a low physical work capacity in the sitting position.

It was considered of especial interest in the present investigation to study whether the estimated degree of severity of the disease had any relationship — and if so to what extent — with the physical work capacity as observed in these tests. It was also of great interest to determine the degree of influence of a number of given factors in the case history of the asthma patient on the work capacity as estimated by the function variables W_{130} and W_{max}. The ranges of values for the physical work capacity in the asthma material may be regarded as an expression among other things, of constitution and the effects of the disease. In comparing the asthma patients with the persons in the control material the two function variables W_{130} and W_{max} were systematically studied. For these two variables — in contrast to W_{150}, for example — values were obtained for almost all patients. It was of particular importance on comparing different groups within the asthma series to be able to base these comparisons on function variables for which a value had been obtained for the majority of the patients.

The mean observed value for the physical work capacity of the patients in the asthma series differed significantly from the mean predicted value. A conceivable reason for this difference was the change in the way of life, from a physical aspect, which the disease appeared to have brought about in the majority of these patients. This assumption was strongly supported by the knowledge gained about these patients on the many occasions of interview and often detailed conversation. This change in their way of living which to no small extent was characterized by the avoidance of physical exertion, was due to many different factors. One such factor was that during periods of symptoms the asthma rendered work difficult or impossible, even when only moderate physical strain was demanded. Another was that during so-called free or almost free intervals, physical strain provoked asthma in several of these patients.

In the asthma series a relationship was found between working capacity and ventilation capacity. A possible causative chain of events leading to this relationship may be that the disease primarily gives a reduction of ventilation capacity which contributes a sedentary way of living and this in turn may result in a low degree of physical exercise and a reduced working capacity.

As in the case of the ventilation

capacity the physical work capacity as observed in the present study appeared to have some relationship with the estimated degree of severity of the disease. For the mean values of both W_{170} and W_{max} there was a significant difference between those patients who since the onset of the disease had attained at least 2000 sickness points and those who had fewer than 1000 points. A relatively large number of case-history factors studied appeared to influence the variable W_{max} to such an extent that on classification of the patients according to sub-groups of these factors a significant difference was found between the groups as regards this variable. Some case history factors also seemed to be of similar importance to the variable W_{170}, but these were fewer in number than for W_{max}.

A significant relationship was found for the patients in the asthma series between ventilation capacity ($FEV_{1.0}$, $FEV_{\%}$, MVV_{40} and MVV_{t}) and work capacity (W_{170} and W_{max}). The coefficient of correlation was about 0.30 (**). The partial correlation between W_{170} and the total number of sickness points — when the influence of the ventilation capacity had been eliminated — was about 0.05, i.e. low. This appears to indicate that the influence of the disease, measured by sickness points, on the physical work capacity is exerted to the greatest part via the influence on the ventilation capacity.

It has been assumed — which is reasonable from a clinical aspect — that the ventilation capacity is of some significance to the physical work capacity. Whether — and if so to what extent — the physical work capacity has any influence on the ventilation capacity cannot be stated with certainty. It seems a reasonable supposition, however, that a low degree of physical exercise resulting in a relatively small muscle mass may have some influence — although probably small — on the ventilation capacity.

In normal adults there is no definite correlation between physical work capacity, determined by the submaximal work test, and vital capacity (Wahlund, 1948, P. O. Åstrand, 1952), ventilation capacity (P. O. Åstrand, 1952, Irma Åstrand, 1960, Grimby and Söderholm, 1963) or the ratio of the residual volume to the total lung capacity (Wahlund, 1948). Dahlström (1957) showed in patients with pulmonary tuberculosis that there was a statistically significant correlation between physical work capacity and both vital capacity and maximal ventilation capacity. No significant relationship was shown, on the other hand, between physical work capacity and the ratio of residual volume to total lung capacity.

It was considered of interest to investigate the conditions in the present series of patients. As in Dahlström's (1957) material, there appeared to be a statistically significant correlation between physical work capacity and both vital capacity and ventilation capacity. As regards the latter the relationship was significant in the asthma series both with the forced expiratory volume per one second and with the maximal ventilation capacity. The relationship between physical work

The *physical work capacity* of the patients in the asthma material was determined *in order to obtain* a quantitative *assessment of the respiratory and circulatory* functional impairment in particular directly or indirectly caused by the disease.

Work tests as methods of clinical investigation in pulmonary diseases *have been used previously among* others by Dahlstrom (1955 and 1957) *on patients with pulmonary tuberculosis*, and by Svanborg (1961) on patients with pulmonary sarcoidosis. Both authors found on an average a low physical work capacity in the sitting position.

It was considered of especial interest in the present investigation to study whether the estimated degree of severity of the disease had any relationship — and if so to what extent — with the physical work capacity as observed in these tests. It was also of great interest to determine the degree of influence of a number of given factors in the case history of the asthma patient on the work capacity as estimated by the function variables W_{170} and W_{max}. The ranges of values for the physical work capacity in the asthma material may be regarded as an expression among other things, of constitution and the effects of the disease. In comparing the asthma patients with the persons in the control material the two function variables W_{130} and W_{max} were systematically studied. For these two variables — in contrast to W_{170}, for example — values were obtained for almost all patients. It was of particular importance on comparing different groups within the asthma series to be able to base these comparisons on function variables for which a value had been obtained for the majority of the patients.

The mean observed value for the physical work capacity of the patients in the asthma series differed significantly from the mean predicted value. A conceivable reason for this difference was the change in the way of life from a physical aspect which the disease appeared to have brought about in the majority of these patients. This assumption was strongly supported by the knowledge gained about these patients on the many occasions of interview and often detailed conversation. This change in their way of living, which to no small extent was characterized by the avoidance of physical exertion was due to many different factors. One such factor was that during periods of symptoms the asthma rendered work difficult or impossible even when only moderate physical strain was demanded. Another was that during so-called free or almost free intervals, physical strain provoked asthma in several of these patients.

In the asthma series a relationship was found between working capacity and ventilation capacity. A possible causative chain of events leading to this relationship may be that the disease primarily gives a reduction of ventilation capacity which contributes a sedentary way of living and this in turn may result in a low degree of physical exercise and a reduced working capacity.

As in the case of the ventilation

capacity the physical work capacity as observed in the present study appeared to have some relationship with the estimated degree of severity of the disease. For the mean values of both W_{130} and W_{max} there was a significant difference between those patients who since the onset of the disease had attained at least 2000 sickness points and those who had fewer than 1000 points. A relatively large number of case-history factors studied appeared to influence the variable W_{max} to such an extent that on classification of the patients according to sub-groups of these factors a significant difference was found between th groups as regards this variable Some case-history factors also seemed to be of similar mportance to the variable W_{130}, but these were fewer in number than for W_{max}.

A significant relationship was found for the patients in the asthma series between ventilation capacity ($FEV_{1.0}$, $FEV_{\%}$, MVV_{40} and MVV_{i}) and work capacity (W_{130} and W_{max}) The coefficient of correlation was about 0.30 ( ) The partial correlation between W_{130} and the total number of sickness points — when the influence of the ventilation capacity had been elimin ted — was bout 0.05, i.e. low This ppears to indicate that the influence of the disease measured by sickness points, on the physical work capacity is exerted to the greatest part via the influence on the ventilation capacity

It ha been ssumed — which is reasonable from a clinical aspect — that the ventilation capacity is of some significance to the physical work capacity Whether — and if so to what extent — the physical work capacity has any influence on the ventilation capacity cannot be stated with certain ty It seems a reasonable supposition, however that a low degree of physical exercise resulting in a relatively small muscle mass may have some influence — although probably small — on the ventilation capacity

In normal adults there is no definite correlation between physical work capacity determined by the submax imal work test, and vital capacity (Wahlund, 1948, P O Åstrand, 1952) ventilation capacity (P O Åstrand, 1952, Irma Åstrand, 1960 Grimby and Söderholm, 1963) or the ratio of the residual volume to the total lung capacity (Wahlund, 1948) Dahlström (1957) showed in patients with pulmonary tuberculosis that there was a statistically significant correlation between physical work capacity and both vital capacity and maximal ventilation capacity No significant relationship was shown, on the other hand, between physical work capacity and the ratio of resid al volume to total lung capacity

It was considered of interest to investigate the conditions in the present series of patients. As in Dahlström's (1957) material, there appeared to be a statistically significant correlation between physical work capacity and both vital capacity and ventilation capacity As regards the latter the relationship was significant in the asthma series both with the forced expiratory volum per one second and with the maximal ventilation capacity The re lation hip between physical work

The *physical work capacity* of the patients in the asthma material was determined in order to obtain a quantitative assessment of the respiratory and circulatory functional impairment in particular directly or indirectly caused by the disease.

Work tests as methods of clinical investigation in pulmonary diseases have been used previously among others by Dahlström (1955 and 1957) on patients with pulmonary tuberculosis and by Svanborg (1961) on patients with pulmonary sarcoidosis. Both authors found on an average a low physical work capacity in the sitting position.

It was considered of especial interest in the present investigation to study whether the estimated degree of severity of the disease had any relationship — and if so to what extent — with the physical work capacity as observed in these tests. It was also of great interest to determine the degree of influence of a number of given factors in the case history of the asthma patient on the work capacity as estimated by the function variables W_{130} and W_{max}. The ranges of values for the physical work capacity in the asthma material may be regarded as an expression, among other things of constitution and the effects of the disease. In comparing the asthma patients with the persons in the control material the two function variables W_{130} and W_{max} were systematically studied. For these two variables — in contrast to W_{150}, for example — values were obtained for almost all patients. It was of particular importance on comparing different groups within the asthma series to be able to base these comparisons on function variables for which a value had been obtained for the majority of the patients.

The mean observed value for the physical work capacity of the patients in the asthma series differed significantly from the mean predicted value. A conceivable reason for this difference was the change in the way of life, from a physical aspect which the disease appeared to have brought about in the majority of these patients. This assumption was strongly supported by the knowledge gained about these patients on the many occasions of interview and often detailed conversation. This change in their way of living which to no small extent was characterized by the avoidance of physical exertion, was due to many different factors. One such factor was that during periods of symptoms the asthma rendered work difficult or impossible, even when only moderate physical strain was demanded. Another was that during so-called free or almost free intervals, physical strain provoked asthma in several of these patients.

In the asthma series a relationship was found between working capacity and ventilation capacity. A possible causative chain of events leading to this relationship may be that the disease primarily gives a reduction of ventilation capacity which contributes a sedentary way of living and this in turn may result in a low degree of physical exercise and a reduced working capacity.

As in the case of the ventilation

capacity the physical work capacity as observed in the present study appeared to have some relationship with the estimated degree of severity of the disease. For the mean values of both W_{170} and W_{max} there was a significant difference between those patients who since the onset of the disease had attained at least 2000 sickness points and those who had fewer than 1000 points. A relatively large number of case-history factors studied appeared to influence the variable W_{max} to such an extent that on classification of the patients according to sub-groups of these factors a significant difference was found between the groups as regards this variable. Some case-history factors also seemed to be of similar importance to the variable W_{170}, but these were fewer in number than for W_{max}.

A significant relationship was found for the patients in the asthma series between ventilation capacity ($FEV_{1.0}$, $FEV_{\%}$, MVV_{40} and MVV_{f}) and work capacity (W_{170} and W_{max}). The coefficient of correlation was about 0.30 ( ). The partial correlation between W_{170} and the total number of sickness points — when the influence of the ventilation capacity had been eliminated — was about 0.03, i.e. low. This appears to indicate that the influence of the disease, measured by sickness points, on the physical work capacity is exerted to the greatest part via the influence on the ventilation capacity.

It has been assumed — which is reasonable from a clinical aspect — that the ventilation capacity is of some significance to the physical work capacity. Whether — and if so to what extent — the physical work capacity has any influence on the ventilation capacity cannot be stated with certainty. It seems a reasonable supposition, however, that a low degree of physical exercise resulting in a relatively small muscle mass may have some influence — although probably small — on the ventilation capacity.

In normal adults there is no definite correlation between physical work capacity, determined by the submaximal work test, and vital capacity (Wahlund, 1948, P. O. Åstrand, 1952), ventilation capacity (P. O. Åstrand, 1952, Irma Åstrand, 1960, Grimby and Söderholm, 1963) or the ratio of the residual volume to the total lung capacity (Wahlund, 1948). Dahlström (1957) showed in patients with pulmonary tuberculosis that there was a statistically significant correlation between physical work capacity and both vital capacity and maximal ventilation capacity. No significant relationship was shown, on the other hand, between physical work capacity and the ratio of residual volume to total lung capacity.

It was considered of interest to investigate the conditions in the present series of patients. As in Dahlström's (1957) material, there appeared to be a statistically significant correlation between physical work capacity and both vital capacity and ventilation capacity. As regards the latter the relationship was significant in the asthma series both with the forced expiratory volume per one second and with the maximal ventilation capacity. The relationship between physical work

capacity and the ratio of residual volume to total lung capacity on the other hand appeared to be negligible this was also in agreement with Dahlström's findings in tuberculous patients

In the asthma series however the ratio RV/TLC showed on an average, a relatively large deviation from the predicted value, in an unfavourable direction It would not have been unexpected if this variable had been found to be of significant importance to the physical work capacity in the present series of patients

Large variations in the degree of severity of the asthma were also found in the patients studied with regard to possible secondary effects of the disease on cardiac function and pulmonary circulation As in the study of the respiratory function and physical work capacity the patients were divided into groups according to the estimated degree of severity of the disease. The findings in the hemodynamic investigations were compared in the different groups Mutual comparisons such as these were of especial value since a sufficiently large control material extending over the whole age range of 30—60 years was not available

The blood pressures in the pulmonary artery and in the lung capillaries were normal during a resting state in all patients studied The flow resistance of the pulmonary vessels (as measured by R units i e. the pressure gradient in mm Hg per blood flow in litres/min) showed a value of less than 2 during the resting state for all patients with the exception of five men and seven women in whom the value exceeded 2 but did not reach 3 units. No pulmonary hypertension or noteworthy increase of the pulmonary vascular resistance need thus necessarily occur during a resting state even in patients with prolonged and severe bronchial asthma The disease in this series of patients therefore differed, as regards the effect on the pulmonary circulation from the chronic obstructive pulmonary diseases studied in the series of patients from other countries which were found to have a tendency to pulmonary hypertension. One possible explanation for this difference is that the patients in the present investigation represent the course in uncomplicated bronchial asthma while in the other series the conditions were affected by a varying collection of such factors as chronic infection air pollution and sometimes cigarette smoking. It seems reasonable therefore to draw the conclusion that even prolonged and relatively severe bronchial asthma does not as a rule lead to pulmonary hypertension at rest if it is not complicated by other conditions giving rise to lung tissue destruction. The series of patients reported here showed a tendency to an increase in the ratio of the residual volume to the total lung capacity but the absence of pulmonary hypertension appears to indicate that this was not due to extensive destructive pulmonary emphysema but only to a less complicated state of hyperinflation

The change in the pulmonary arterial pressure during physical exercise on the other hand exhibited a relationship with the estimated degree of severity of the disease — measured by

sickness points — in this group of patients. This relationship was statistically significant for the intensity of the disease during the five years almost immediately preceding the time of the investigation. If on the contrary the over all degree of severity was taken into account, no significant tendency to a higher rise in pressure on increase in the number of sickness points was found. This supports the view that bronchial asthma that has been intensive for a few recent years does influence the pulmonary circulation even during a symptom free state, at least during physical exercise as shown by rise in pressure this has not been shown during a resting state. It also may indicate that the deterioration is reversible i.e. it subsides when the disease becomes less active.

The effect of anoxic anoxia on the pulmonary circulation in man has been studied in normal persons by several authors including Motley Cournand, Werkö, Himmelstein and Dresdale 1947) Westcott, Fowler Scott, Hauenstein and McGuire (1951) Asmussen and Nielsen (1955) Doll, Valentin and Tietz (1957) Fritts Harris Clauss Odell and Cournand (1958) and Fishman Fritt and Cournand (1960) and also in a few investigations on patients with chronic pulmonary disease (Dexter Whittenberger Gorlin, Lewis, Haynes and Spiegl 1951 and Fishman, McClement, Himmelstein and Cournand, 1952). Of patients with chronic pulmonary diseases about 1/3 appear to react to slight anoxic anoxia (about 16 % in FI_{O_2}) with a significant increase in the pulmonary arterial pressure while the cardiac output is unchanged or somewhat increased (Fishman et al. 1952). More pronounced anoxic anoxia i.e. leading to arterial oxygen saturation of less than 80 % gives rise, however in the majority of patients with pulmonary emphysema to a considerable increase in the cardiac minute volume (Wade and Bishop 1962). For normal subjects Fritts et al. (1958) and Fishman et al (1960) reported that during the breathing of 12 % oxygen the cardiac output under steady state conditions increased by about 20 % in comparison with the normal resting conditions. No studies of the effect of anoxic anoxia on patients with bronchial asthma of the type present in the patients reported here appear however to have been made. In the present investigations the patients were submitted to fairly marked anoxic anoxia (11 % FI_{O_2} mean arterial *oxygen saturation of 70 %*). The pulmonary arterial pressure thereby rose significantly but only relatively moderately no significant change in the cardiac output was shown. Neither was any noteworthy difference observed between groups in which the asthma had shown different degrees of severity. Two patients who during anoxic anoxia developed *transient but considerable* pulmonary hypertension *did not show any deviation from* the general tendency *in the rest of the material towards* an insignificant influence *on* the cardiac output by the *anoxia. It is true that the* pressure *in the lung capillaries* was not *recorded during the anoxic anoxia, and therefore* the possibility of a small increase *in the pulmonary* capillary pressure cannot be precluded. Assuming this

capacity and the ratio of residual volume to total lung capacity on the other hand appeared to be negligible this was also in agreement with Dahlström s findings in tuberculous patients

In the asthma series, however the *ratio RV/TLC showed* on an average a relatively large deviation from the predicted value in an unfavourable direction It would not have been unexpected if this variable had been found to be of significant importance to the physical work capacity in the present series of patients

Large variations in the degree of severity of the asthma were also found in the patients studied with regard to possible secondary effects of the disease on *cardiac function* and *pulmonary circulation* As in the study of the respiratory function and physical work capacity the patients were divided into groups according to the estimated degree of severity of the disease The findings in the hemodynamic investigations were compared in the different groups. Mutual comparisons such as these were of especial value since a sufficiently large control material extending over the whole age range of 30—60 years was not available.

The blood pressures in the *pulmonary artery* and in the *lung capillaries* were normal during a resting state in all patients studied. The flow resistance of the pulmonary vessels (as measured by R units, i e the pressure gradient in mm Hg per blood flow in litres/min) showed a value of less than 2 during the resting state for all patients with the exception of five men and seven women in whom the value exceeded 2 but did not reach 3 units. No pulmonary hypertension or noteworthy increase of the pulmonary vascular resistance need thus necessarily occur during a resting state even in patients with prolonged and severe bronchial asthma. The disease in this series of patients therefore differed as regards the effect on the pulmonary circulation from the chronic obstructive pulmonary diseases studied in the series of patients from other countries which were found to have a tendency to pulmonary hypertension. One possible explanation for this difference is that the patients in the present investigation represent the course in uncomplicated bronchial asthma, while in the other series the conditions were affected by a varying collection of such factors as chronic infection, air pollution and sometimes cigarette smoking It seems reasonable therefore to draw the conclusion that even prolonged and relatively severe bronchial asthma does not as a rule lead to pulmonary hypertension at rest if it is not complicated by other conditions giving rise to lung tissue destruction. The series of patients reported here showed a tendency to an increase in the ratio of the residual volume to the total lung capacity but the absence of pulmonary hypertension appears to indicate that this was not due to extensive destructive pulmonary emphysema but only to a less complicated state of hyperinflation.

The change in the pulmonary arterial pressure during physical exercise, on the other hand, exhibited a relationship with the estimated degree of severity of the disease — measured by

sickness points — in this group of patients. This relationship was statistically significant for the intensity of the disease during the five years almost immediately preceding the time of the investigation. If on the contrary the over-all degree of severity was taken into account, no significant tendency to a higher rise in pressure on increase in the number of sickness points was found. This supports the view that bronchial asthma that has been intensive for a few recent years does influence the pulmonary circulation even during a symptom free state, at least during physical exercise, as shown by rise in pressure this has not been shown during a resting state. It also may indicate that the deterioration is reversible, i. e. it subsides when the disease becomes less active.

The effect of anoxic anoxia on the pulmonary circulation in man has been studied in normal persons by several authors, including Motley Cournand, Werkö Himmelstein and Dresdale (1947) Westcott, Fowler Scott Hauenstein and McGuire (1951) Asmussen and Nielsen (1955) Bolt, Valentin and Tietz (19 7) Fritts, Harris, Clauss, Odell and Cournand (1958) and Fishman Fritt and Cournand (1960) and also in a f w in estigations on patients with chronic pulmonary disease (Dexter Whittenberger Gorlin, Lewis, Haynes and Spiegl, 1951 and Fishman, McClement, Himmelstein and Cournand, 1952) Of patients with chronic pulm nary diseases, about 1/3 appear to react to slight anoxic anoxia (about 16 In FI_{O_2}) with a significant increase in the pulmonary arterial pressure while the cardiac output is unchanged or somewhat increased (Fishman et al 1952) More pronounced anoxic anoxia, i. e. leading to arterial oxygen saturation of less than 80 %, gives rise, however in the majority of patients with pulmonary emphysema to a considerable increase in the cardiac minute volume (Wade and Bishop 1962) For normal subjects, Fritts et al. (1958) and Fishman et al (1960) reported that during the breathing of 12 % oxygen the cardiac output under steady state conditions increased by about 20 % in comparison with the normal resting conditions. No studies of the effect of anoxic anoxia on patients with bronchial asthma of the type present in the patients reported here appear however to have been made. In the present investigations the patients were submitted to fairly marked anoxic anoxia (11 % FI_{O_2}, mean arterial oxygen saturation of 76 /s) The pulmonary arterial pressure thereby rose significantly but only relatively moderately no significant change in the cardiac output was shown. Neither was any noteworthy difference observed between groups in which the asthma had shown different degrees of severity Two patients who during anoxic anoxia developed transient but considerable pulmonary hypertension did not show any deviation from the general tendency in the rest of the material towards an insignificant influence on the cardiac output by the anoxia. It is true that the pressure in the long capillaries was not recorded during the anoxic anoxia, and therefore the possibility of a small increase in the pulmonary capillary pressure cannot be precluded. Assuming this

capacity and the ratio of residual volume to total lung capacity on the other hand appeared to be negligible this was also in agreement with Dahlstrom's findings in tuberculous patients

In the asthma series however the ratio RV/TLC showed, on an average, a relatively large deviation from the predicted value, in an unfavourable direction It would not have been unexpected if this variable had been found to be of significant importance to the physical work capacity in the present series of patients.

Large variations in the degree of severity of the asthma were also found in the patients studied with regard to possible secondary effects of the disease on cardiac function and pulmonary circulation As in the study of the respiratory function and physical work capacity the patients were divided into groups according to the estimated degree of severity of the disease. The findings in the hemodynamic investigations were compared in the different groups Mutual comparisons such as these were of especial value since a sufficiently large control material extending over the whole age range of 30—60 years was not available.

The blood pressures in the pulmonary artery and in the lung capillaries were normal during a resting state in all patients studied. The flow resistance of the pulmonary vessels (as measured by R units i e. the pressure gradient in mm Hg per blood flow in litres/min) showed a value of less than 2 during the resting state for all patients with the exception of five men and seven women in whom the value exceeded 2 but did not reach 3 units No pulmonary hypertension or noteworthy increase of the pulmonary vascular resistance need thus necessarily occur during a resting state even in patients with prolonged and severe bronchial asthma The disease in this series of patients therefore differed, as regards the effect on the pulmonary circulation from the chronic obstructive pulmonary diseases studied in the series of patients from other countries which were found to have a tendency to pulmonary hypertension. One possible explanation for this difference is that the patients in the present investigation represent the course in uncomplicated bronchial asthma, while in the other series the conditions were affected by a varying collection of such factors as chronic infection air pollution and sometimes cigarette smoking It seems reasonable therefore to draw the conclusion that even prolonged and relatively severe bronchial asthma does not as a rule lead to pulmonary hypertension at rest if it is not complicated by other conditions giving rise to lung tissue destruction The series of patients reported here showed a tendency to an increase in the ratio of the residual volume to the total lung capacity but the absence of pulmonary hypertension appears to indicate that this was not due to extensive destructive pulmonary emphysema but only to a less complicated state of hyperinflation.

The change in the pulmonary arterial pressure during physical exercise, on the other hand, exhibited a relationship with the estimated degree of severity of the disease — a ...

explained by the lower oxygen uptake capacity of the asthma patients (partly due to the difference in sex proportion) and does not necessarily indicate an abnormal change of circulatory function. The average arterio-venous oxygen difference per heart rate was on the other hand approximately the same for the asthma patients and the control material, although the individual variation was rather high. The asthma patients may therefore from this point of view be regarded as having a "normokinetic circulation. During muscular work, the cardiac output in relation to oxygen uptake was also found to increase similarly in the asthma patients and in the normal groups. At rest, however the cardiac output per oxygen uptake was relatively low in the asthma patients this may possibly depend on the difference in age (Granath, Jonsson and Strandell, 1961) the asthma patients being older than the control materials. For comparison Holmgren, Jonsson, Linderholm, Sjöstrand and Ström (1958) and Donald, Bishop and Wade (1954) found that a proportion of patients with mitral stenosis, in sinus rhythm were "hypokinetic i they exhibited both low cardiac minute volume in relation t the oxygen uptake and a high arterio-venous oxygen difference in relation to the heart rate On the other hand, the opposite condition, i. e a hyperkinetic circul tion, held for person with so-called vasoregulatory thenia (Holmgren et al., 1957) These individual thus showed a high cardiac minut volum but a low peripheral utilization of the oxygen content.

In a group of older normal men, Granath et al. (1961) found a mean arterio-venous oxygen differenc in relation to oxygen uptake that was in closer agreement with the mean value for the asthma series. The significance of age is appreciable by the fact that regardless of the estimated degree of severity of the disease the patients in the asthma series with a higher mean age had a somewhat higher a—v O_2 difference in relation to oxygen uptake than the patients with a lower mean age Increasing age is also accompanied by a decrease of the maximal heart rate level and this change may be accompanied by a tendency to increased a—v O_2 difference in relation to heart rate.

For each of the function variables, such as for example the pressure in the pulmonary artery the group of patients showing the largest deviation from the normal values were selected. Pairs of variables were then studied to find out how many patients were common to the two groups. It was found that for the mean pressure in the pulmonary artery during anoxic anoxia, and the arterial saturation also during anoxic anoxia, a larger number of patients than predicted were common to the two groups. The difference between the observed and predicted value was significant. A similar significant difference was also found for both combinations, when the mean pressure in the pulmonary artery during exercise was paired firstly with the mean pressure in the pulmonary artery during anoxic anoxia and secondly with the pulmonary vascular resistance during

pressure to be unchanged, the mean pulmonary vascular resistance for the series as a whole is then *higher during* the anoxia than during a normal resting state. This increase in resistance may be interpreted as being due to alveolar hypoxia

The physical work capacity was found on the whole to be the same in the supine as in the sitting position which is in agreement with the findings of Holmgren, Jonsson and Sjöstrand (1960) in normal subjects. Patients with pulmonary sarcoidosis (Svanborg 1961) on the other hand like orthostatic patients, exhibited a higher capacity in the supine than in *the sitting position* The fact that in the asthma series the heart rate at certain loads was practically the same in the supine and the sitting position, and that the calculated mean work load in *the supine and sitting positions at heart* rates of 130 and 150 was approximately the same, supports the view that the orthostatic effect in the sitting position was not of any noteworthy *order of size in these patients* In the orthostatic test also the mean increase in heart rate was found to be of a normal order of size.

For the stroke volume at rest no notable difference was *found in the* present material on classification into groups according to the estimated degree of severity of the disease During exercise at 300 kpm/min for men and *200 kpm/min for women,* the stroke volume increased in comparison with the resting conditions by an average of 25 % for the former and 16 % for the latter The increase was more marked than *that reported* previously for normal persons (Bevegård, Holmgren and Jonsson, 1960 and Holmgren et al 1960) but the latter persons were on an average younger The tendency in the asthma patients to a relatively large *increase in the stroke* volume on changing over from a resting state to exercise is more similar to the results published in a preliminary report by Granath, Jonsson and Strandell (1961) *These results* were based on a material of normal men of ages 60—83 years The differences between the asthma series and the control materials lie essentially in the resting values. *The asthma* material had a lower stroke volume at rest both absolute and in relation to body size. This was possibly due to an inadequate distribution of the blood volume When exercise was commenced redistribution probably took place, with the result *that both the central blood volume* and the stroke volume increased. One factor possibly contributing to the significantly increased stroke volume during the first work load was an increase *in myocardial "contractility* An effect such as this could not be precluded in the asthma series

It is of interest to analyse the "circulatory efficiency" of the asthma *patients, i e cardiac output* in relation to oxygen need and *heart rate* The patients in the present series showed on an average a higher arterio-venous oxygen difference in relation to oxygen uptake than that reported for a group of normal individuals (most of whom were males) (*Holmgren, Jonsson,* Levander Linderholm Sjostrand and Ström 1957 *Holmgren et al. 1960* Bevegård et al 1960) This may be

explained by the lower oxygen uptake capacity of the asthma pati ents (partly due to the difference in sex proportion) and does not necessarily indicate an abnormal change of circulatory function. The average arterio-venous oxygen dif ference per heart rate was on the other hand approximately the same for the asthma patients and the control ma terial, although the individual variat ion was rather high. The asthma patients may therefore from this point of view be regarded as having a "normokinetic" circulation. During muscular work, the cardiac output in relation to oxygen uptake was also found to increase similarly in the asthma patients and in the normal groups. At rest, however the cardiac output per oxygen uptake was relatively low in the asthma patients this may possibly depend on the difference in age (Granath, Jonsson and Strandell, 1961) the asthma patients being older than the control materials. For comparison H lmgren, Jonsson, Linderholm, Sjöstrand and Ström, (1958) and Donald, Bishop and Wade (1954) found that a proportion of patients with mitral stenosis, in sinus rhythm, were "hypokinetic i. e they exhibited both a low cardiac minute volume in rela tion to the oxygen uptake and a high arterio-venous o ygen difference in relation to the heart rat On the other hand, the opposit condition, i. e a "hyperkinetic" circulation, held for persons with so-called vasoregulatory asthenia (Holmgren et L. 1937) These indi idual thus showed a high cardiac minut ol me but a low peripheral utilization of the oxygen content.

In a group of older normal men, Granath et al. (1961) found a mean arterio-venous oxygen difference in relation to oxygen uptake that was in closer agreement with the mean value for the asthma series. The significance of age is appreciable by the fact that regardless of the estimated degree of severity of the disease the patients in the asthma series with a higher mean age had a somewhat higher a—v O_2 difference in relation to oxygen uptake than the patients with a lower mean age. Increasing age is also ac companied by a decrease of the maxi mal heart rate level, and this change may be accompanied by a tendency to increased a—v O_2 difference in rela tion to heart rate.

For each of the function variables, such as for example the pressure in the pulmonary artery the group of patients showing the largest deviation from the normal values were selected. Pairs of variables were then studied to find out how many patients were com mon to the two groups. It was found that for the mean pressure in the pul monary artery during anoxic anoxia and the arterial saturation also during anoxic anoxia, a larger number of patients than predicted were common to the two groups. The difference between the observed and predicted value was significant. A similar significant dif ference was also found for both com binations, when the mean pressure in the pulmonary artery during exercise was paired firstly with the mean pressure in the pulmonary artery during anoxic anoxia and secondly with the pulmonary vascular resistance during

exercise For practically all combinations a higher number of patients common to the two variables was observed than predicted, even if the difference was not significant. The variable that showed the lowest mean correlation with the other variables was RV/TLC, and that showing the next lowest was $FEV_{1.0}$. In consideration of the comparatively high increase in the ratio of the residual volume to the total lung capacity shown on an average by these patients, a greater degree of correlation between this variable and the rest might possibly have been expected

Hurtado Kaltreider Fray Brooks and McCann (1934) and Borden Wilson Ebert and Wells (1950) maintained that an increased RV/TLC ratio was an index of the degree of severity of emphysema Herschfus, Bresnick and Segal (1953) found that according to this index all the patients in their material exhibited pronounced emphysema. It was pointed out however that to judge by the arterial oxygen saturation for example which among other factors was regarded by Baldwin et al. (1949) as particularly significant in this respect the patients only had moderate emphysema In the present series the relationship between a high RV/TLC ratio and the pulmonary vascular resistance during exercise was not significant This supported the view that in the patients in this series the degree of hyperinflation complicated by alveolar tissue destruction was only small or moderate this is presuming that a relationship exists between pulmonary vascular resistance and emphysema — if by emphysema is meant a condition of inflation complicated by alveolar wall destruction

The hemodynamic findings in the patients in this asthma series were on the whole only slightly abnormal This applied especially to the material as a whole, but also to the group of patients who according to the case history had been most severely affected. It was found also that it was not always in the patients who had had especially severe symptoms that the values showed the greatest difference from the normal values. It was therefore often of interest to make a separate study of those patients in whom this difference was found to be greatest This group however exhibited a status that in many respects could be regarded as remarkably good in consideration of the fact that this material was composed of persons who during often large parts of their lives had been completely or partly disabled by asthma symptoms It therefore seemed reasonable to attribute the relatively small extent of the abnormal deviations in the hemodynamic findings to the fact that the dilatation of the terminal bronchioles indicated by the increased residual volume was not followed by destruction in the bronchial walls of such a degree as to cause irreversible damage. The reason that in these asthma patients, in spite of the often greatly increased residual volume there did not appear to be any great functional consequences of the disease was assumed to be that the material did not include patients in whom the asthma was complicated by chronic bronchitis. It is considered probable that when bronchial asthma is com

plicated by chronic bronchitis there is a great increase in the risk of emphysema as defined by the American Thoracic Society (1962) "Emphysema is an anatomical alteration of the lung characterized by an abnormal enlargement of the air spaces distal to the terminal, nonrespiratory bronchioles, accompanied by destructive changes of the alveolar walls" or the similar definition earlier proposed by the World Health Organization Committee on Chronic Cor Pulmonale (1961) Since it was considered essential to emphasize the difference between alveolar dilatation and "alveolar wall destruction the former definitions were preferred — like Fletcher Hugh Jones, McNicol and Pride (1963) did — to that given by the Ciba Guest Symposium (1959) "Emphysema is a condition of the lung characterized by increase beyond the normal in the size of air spaces distal to the terminal bronchioles either from dilatation or from destruction of their walls

Fletcher et al. (1963) made this distinction because alveolar dilatation was regarded as reversible, in contrast to irreversible destructive changes. The present investigations, however have offered some support for the view that in patients with bronchial asthma hyperinflation does not appear to be characterized by reversibility to any great extent, but that th condition is probably less complicated than that present in emphysema. It i therefore from thi point of view considered reasonable to distinguish between hyperinflation and emphysema in a material such as the present one also.

An ttempt was made to form an idea of the influence exerted on the hemodynamic functions by different factors representing the estimated degree of severity of the asthma. The values for each of certain different functions, e. g. the pressure in the pulmonary artery were studied in subgroups of each of these different factors, e. g the number of sickness points. To obtain the best contrast two extreme groups were selected the first comprised patients who showed the greatest difference from the normal values in a pathological direction, and the second in the opposite direction. Each extreme group comprised 20 patients (i. e. 36 per cent of the whole series) It was then found that for several of the functions the selected group with the pathological deviation had a significantly higher age than the other group. The former group as most of the older patients, also often showed a relatively high age at onset of the disease. It seems possible but cannot be stated with certainty that age had a deterious influence on the functional sequelae as regards the pulmonary circulation in this series of patients. On analysing the significance of different case history factors for some indices of ventilation capacity and lung volumes, it was found that age was on an average of greater influence in this respect among the asthma patients than among the control persons. This offers some support for the view that the consequences of bronchial asthma are of greater functional significance in older than in younger persons. The resistance of the tissues to the strain produced by asthma probably decreases with in-

exercise For practically all combinations a higher number of patients common to the two variables was observed than predicted even if the difference was not significant The variable that showed the lowest mean correlation with the other variables was RV/TLC, and that showing the next lowest was $FEV_{1.0}$ In consideration of the comparatively high increase in the ratio of the residual volume to the total lung capacity shown on an average by these patients a greater degree of correlation between this variable and the rest might possibly have been expected

Hurtado Kaltreider Fray Brooks and McCann (1934) and Borden Wilson Ebert and Wells (1950) maintained that an increased RV/TLC ratio was an index of the degree of severity of emphysema Herschfus Bresnick and Segal (1953) found that according to this index all the patients in their material exhibited pronounced emphysema It was pointed out, however that to judge by the arterial oxygen saturation, for example, which among other factors was regarded by Baldwin et al (1949) as particularly significant in this respect the patients only had moderate emphysema In the present series the relationship between a high RV/TLC ratio and the pulmonary vascular resistance during exercise was not significant. This supported the view that in the patients in this series the degree of hyperinflation complicated by alveolar tissue destruction was only small or moderate this is presuming that a relationship exists between pulmonary vascular resistance and emphysema — if by emphysema is meant a condition of inflation complicated by alveolar wall destruction.

The hemodynamic findings in the patients in this asthma series were on the whole only slightly abnormal This applied especially to the material as a whole but also to the group of patients who according to the case history had been most severely affected It was found also that it was not always in the patients who had had especially severe symptoms that the values showed the greatest difference from the normal values It was therefore often of interest to make a separate study of those patients in whom this difference was found to be greatest This group, however exhibited a status that in many respects could be regarded as remarkably good in consideration of the fact that this material was composed of persons who during often large parts of their lives had been completely or partly disabled by asthma symptoms It therefore seemed reasonable to attribute the relatively small extent of the abnormal deviations in the hemodynamic findings to the fact that the dilatation of the terminal bronchioles indicated by the increased residual volume was not followed by destruction in the bronchial walls of such a degree as to cause irreversible damage The reason that in these asthma patients in spite of the often greatly increased residual volume there did not appear to be any great functional consequences of the disease was assumed to be that the material did not include patients in whom the asthma was complicated by chronic bronchitis. It is considered probable that when bronchial asthma is com

ed a significant decrease in the mean pressure in the pulmonary artery while the cardiac minute volume was essentially unchanged. The effect of breathing oxygen in patients with chronic bronchitis, some of whom had pulmonary emphysema has been studied by Coles, Pisa and Thomas (1963). These authors found a tendency to a lower mean pressure and a reduced cardiac output during oxygen breathing than during the breathing of air.

In the present investigations a moderate mean increase in the cardiac minute volume was obtained both during hyperoxic breathing and during acetylcholine infusion, in comparison with ordinary resting conditions. The pressure conditions in the pulmonary artery remained essentially unchanged, which was to be expected since the material consisted of normotensive patients.

During hypercapnoea a moderate mean increase in both the cardiac minute volume and the pressure in the pulmonary artery was recorded, in comparison with ordinary resting conditions. The results cannot be claimed with certainty however to support the view that pulmonary vasoconstriction was provoked.

Summary

There appear to have been no systematic studies made previously on the effects of uncomplicated bronchial asthma on the cardiac and pulmonary functions. By uncomplicated bronchial asthma is meant that there is no evidence that chronic bronchitis — which condition has been defined — is simultaneously present or has previously been present. Neither does there appear to have been any study made previously in which a systematic attempt was made to assess the relationship between different factors characteristic of the case history of an asthma patient and the status exhibited by a group of such patients with regard to ventilation capacity lung volumes, physical work capacity and hemodynamic function.

The results are given of an investigation (carried out mainly *during the* period August 1961—March 1963) on the long term effects of bronchial asthma on the respiratory and cardiovascular functions in a series of patients who had lived in a given region during a certain period, and who during this period had been admitted to hospital in Uppsala for treatment of this disease. Of the 128 patients studied, 12 were diagnosed as having or having had — in addition to the bronchial asthma — a chronic disease which it was considered might have had a decisive influence on the cardiorespiratory functions. These patients were not included in the general analyses of the results of indices of the different functions studied. Because of the possible influence of the supervening chronic disease this group is reported separately.

The degree of severity of the asthma

creasing age, and in this respect a higher age at onset might be of greater disadvantage than a lower for a given duration of the disease

On studying the influence of different factors in the case history on the functional status at the time of the present investigation, certain findings appeared to indicate that the influence of the asthma on some essential functions was of a reversible nature. In order to study this question further a group of patients with a relatively high disease intensity during the years almost immediately preceding the time of the investigation was compared with a group in which the intensity of the disease during the same period was relatively low Comparison was also made between a group of patients with a high over all degree of severity and a group in which this was low The first comparison but not the second, showed a significant difference between the two groups as regards the function variable studied

In the present investigations on pulmonary arterial blood pressure and pulmonary circulation it was seen that during resting conditions this series of patients exhibited a status that differed to only a relatively small extent from normal values. To a certain — but considerably lesser — extent this may also be said to hold for these function variables during physical exercise. In order to further provoke — in addition to the graded work test — any possible abnormal reactions that were not revealed during a resting state the anoxic anoxia test was carried out In a further effort to illustrate the consequences of bronchial asthma for this series of patients, pressure and flow studies were made during heart catheterization (to be published) on a randomly selected number of patients from the series in 58 patients during the inspiration of a mixture of 50 % oxygen in nitrogen, in 40 patients during the inspiration of 5 % CO_2 in air and in 19 patients during continuous infusion of acetylcholine into the pulmonary artery at a dose rate of 11 mg/min. Such tests have to be evaluated with caution, as circulatory or respiratory steady state during the period of measurement cannot be guaranteed (for methods, see page 28) — which makes determinations of cardiac output by the Fick principle uncertain. The general circulatory reaction nevertheless seems of interest, and a preliminary summary of the results is therefore given here.

As no studies appear to have been made previously on the effect of hyperoxic breathing acetylcholine infusion or hypercapnoea in patients with uncomplicated bronchial asthma these examinations were considered warranted. The effect of hypercapnoea on the pulmonary circulation in patients with pulmonary emphysema has been studied by among others, Fishman, Fritts and Cournand (1960) who found a significant increase in both the cardiac minute volume and the mean pressure in the pulmonary artery during the inspiration of 3—5 % CO_2 in air The effect of an infusion of acetylcholine during a resting state in patients with pulmonary emphysema and pulmonary hypertension has been studied among others, by Behnke, Williams and White (1963) who show

ed a significant decrease in the mean pressure in the pulmonary artery while the cardiac minute volume was essentially unchanged. The effect of breathing oxygen in patients with chronic bronchitis, some of whom had pulmonary emphysema, has been stud ied by Coles, Pisa and Thomas (1963) These authors found a tendency to a lower mean pressure and a reduced cardiac output during oxygen breathing than during the breathing of air

In the present investigations a mod erate mean increase in the cardiac minute olume was obtained both dur ing hyperoxic breathing and during acetylcholine infusion, in comparison with ordinary resting conditions. The pressure conditions in the pulmonary artery remained essentially unchanged, which was to be expected since the material consisted of normotensive patients.

During hypercapnoea a moderate mean increase in both the cardiac minute volume and the pressure in the pulmonary artery was recorded, in comparison with ordinary resting conditions. The results cannot be claimed with certainty however to support the view that pulmonary vasoconstriction was provoked.

Summary

There appear to have been no syst ematic studies made previously on the effects of uncomplicated bronchial asthma on the cardiac and pulmonary functions By uncomplicated bronchial asthma is meant that there is no evidence that chronic bronchitis — which condition has been defined — is imultaneously present or has previously been present. Neither does there appear to have been any study made previously in which a systematic attempt was made to assess the relationship between different factors characteristic of the case hist ory of an asthma patient and the status exhibited by a group of such patient with regard to ventilation capacity lung volumes, physical work capacity and hemodynamic function.

The results are given of an investig ation (carried ut mainly during the period August 1961—March 1963) on the long term effects of bronchial asthma on the respiratory and cardiovascular functions in a series of patients who had lived in a given re gion during a certain period and who during this period had been admitted to hospital in Uppsala for treatment of this disease. Of the 128 patients studied, 12 were diagnosed as having or having had — in addition to the bronchial asthma — a chronic disease which it was considered might have had a decisive influence on the cardiorespiratory functions These patients were not included in the general ana lyses of the results of indices of the different functions studied. Because of the possible influence of the super vening chronic disease, this group is reported separately

The degree of severity of the asthma

8

in the series of patients studied here varied greatly The patients were therefore divided into groups according to the estimated degree of severity of the disease and the investigation findings were then compared between these groups In addition comparisons were also made between the asthma material —or its groups—and control material.

The tests which measure ventilation capacity showed, together with the ratio of the residual volume to the total lung capacity the greatest mean percentage deviations from the predicted value. The smallest deviations were noted for the total lung capacity and the vital capacity There appeared to be some relationship — as expected — between the estimated degree of severity of the disease and the size of both the ratio RV/TLC and the ratio FRC/TLC. In the asthma series on comparison with the control material the mean ratio RV/TLC in particular showed a highly significant difference

The value for the material as a whole differed on an average highly significantly from the predicted value in all of the tests used as indices of the ventilation capacity With respect to each of the tests significant mutual differences were shown between the asthma groups formed according to the estimated degree of severity of the disease i e. there was a significant difference between the mean value for those patients with fewer than 1000 total sickness points and that for the patients with a higher number The value for the material as a whole differed on an average highly significantly from the predicted value (according to the control material) for the ratio of both the residual volume and the functional residual capacity to the total lung capacity

Regression analyses of the importance of different case history factors to the ventilation capacity showed that the intensity of the disease during the five-year period almost immediately preceding the time of the investigation was of relatively great influence compared with the over all severity since the onset of the asthma This appears to support the view that the influence of the disease on the ventilation capacity is of a reversible nature when the disease becomes less active. The regression analysis showed, however that the relation between the ratios of the residual volume and the functional residual capacity to the total lung capacity and the intensity of the disease during the almost immediately previous years was relatively small On the other hand there was a closer relationship between the ratio of the residual volume in particular to the total lung capacity and the over all severity of the disease since the onset of the asthma This suggests that the present study has offered some support for the view that in uncomplicated bronchial asthma hyperinflation does not appear to be characterized by reversibility to any great extent.

The mean value for the observed physical work capacity in the asthma patients differed significantly from the predicted value. It was considered that this might be due to a certain extent to the alteration, from a physical aspect, in the way of living i. e a decrease in the circulatory functional

capacity was produced because of lack of exercise which appeared to have resulted from the disease in the majority of the patients. On classification of the patients into groups according to the estimated degree of severity of the asthma, it was found that among the sub-groups formed with regard to several case history factors there was a significant difference between the mean calculated values for the physical work capacity. The influence of the disease, measured by sickness points, on the physical work capacity appears to be exerted to a great part via the influence on the ventilation capacity.

A significant relationship was found for the patients in the asthma series between, on the one hand, both vital capacity and ventilation capacity as measured by the tests of $FEV_{1.0}$, $FEV\%$, MVV_{40} or MVV_{f} and, on the other hand, physical work capacity as measured by W_{130} or W_{max}. The relationship between physical work capacity and the ratio of residual volume to total lung capacity appeared to be negligible.

No definite signs of cor pulmonale — definition according to WHO Committe on Chronic Cor Pulmonale (1961) — were found in any of the patients in this series. All those on whom heart catheterization was performed had normal pressures in the lesser circulation at rest. The disease in these patients therefore differed, with regard to the pressure conditions in the pulmonary artery, from the series of patients, reported from other countries with chronic obstructive pulmonary disease, and with some tendency to pulmonary hypertension. A probable explanation is that this material is considered to represent the course in uncomplicated bronchial asthma while in the other series of cases various other factors such as chronic infection and air pollution were involved. It seems reasonable to conclude therefore that bronchial asthma does not as a rule lead to pulmonary hypertension if it is not complicated by other diseases leading to lung tissue destruction. The series of patients reported here showed on an average a moderate tendency to an increased ratio of the residual volume to the total lung capacity but since there was no pulmonary hypertension during a normal resting state it was improbable that this meant the presence of extensive destructive pulmonary emphysema. It is more likely that the increase was due only to a slightly complicated state of hyperinflation.

During physical exercise, however, the pressure in the pulmonary artery rose to abnormal values in some patients. The pressure during exercise was found to have some relationship with the estimated degree of severity of the disease. This relationship was significant for the intensity of the disease during the five years almost immediately preceding the time of the investigation. It was not significant, on the other hand, when the over all degree of severity since the onset of the asthma was taken into account. This supports the view that bronchial asthma that has been intensive for a few years does have an effect on the pulmonary circulation even during a symptom free state. This influence was not shown during a resting state, but

only when physical exercise was be gun There appears to be some indica tion that *the pressure increase is of a* reversible nature, i e. it decreases or disappears when the disease becomes less active

The results of the investigation ap peared to show that the orthostatic effect on the circulation in both the standing and the sitting positions as *compared to the supine position* was of a normal order of size in the asthma material

The stroke volume at rest in the asthma patients was on an average somewhat lower compared with nor mal control persons On changing over from a resting state to physical work *the asthma patients showed on an* average a relatively great increase in the stroke volume. The arterio-venous oxygen difference in relation to oxygen *uptake was on an average higher in the* asthma series than in the control material — which mainly consisted of younger male persons. A "hypokinetic circulation was not judged to be present however as on an average the arterio venous oxygen difference in rel ation to heart rate, as *well as the increase of cardiac output in relation* to increase of oxygen uptake during muscular work did not deviate from that of the control *material* The asthma patients were therefore from this point of view regarded as "normo kinetic

The *relationship between pairs of* different function variables was stud ied In the asthma material there ap peared to be some *relationship* between *several of these* variables, and in some pairs this relationship was significant

The function variable which on being paired with others showed the lowest mean correlation was the ratio RV/TLC. In *consideration of the relatively* large increase in this ratio shown on an average by these patients a relat *ionship with other* variables *would* not have been unexpected *In this material* no relationship was found between RV/TLC ratio and the pressure in the pulmonary artery or the pulmonary vascular resistance, *not even when the* two latter were calculated during phys ical exercise. This probably indicates *that in patients with a high RV/TLC* ratio hyperinflation complicated with alveolar tissue destruction occurred only to a small or moderate extent It *is considered possible that the reason* may be found here for the fact that the hemodynamic findings were abnormal only to such a small extent in these *patients. This fact is valid for the* material as a whole, but also to a certain extent for the group of patients in whom the severity of the asthma was *assessed as pronounced.* It was found, however that it was not always the values in patients with a history of particularly severe asthma *that differ* ed *most greatly* from the normal values of the function variables. For this reason the patients who for certain *function variables were found to show* the greatest difference were studied separately This group of patients also exhibited a status that *in several respects* could be regarded as remark ably good considering that this mater ial was composed of patients who dur ing *often great parts of their lives* had been completely or partly disabled by asthma symptoms

VIII SOCIO-MEDICAL ASPECTS

Mortality

Since in this investigation the cli ical course was not sul jected to an ndependent and complete analysis, the nortality study was included in the ocio-medical analysis and discussion. The mortality was studied both for he total asthma series and also for those patients who, on their first admission to hospital, were resident in the Uppsala urban district

Mortality study on the total asthma series i e all those patients who on admission to hospital fulfilled the ori ginal crit ria for incl sion in the ma t rial

For a detailed significance analysis of the mortality the whole of the thma series, i 170 patients, were ncluded. The calculations were made according to the principles described in Statistical Methods (page 158) The expected number of deaths according to the risk figures for Swedish towns was 5.31 for men and 5.00 for women (see Table 48) The risk figures for Swedish towns were used. This implies an approximation be cause only a few more than 80 / of the patients in the asthma series belonged to the town population. The actual number of deaths among the 1 6 cases of the asthma series—i. e. all those who on admission to hospital fulfilled the criteria for inclusion in the material—was 15 (8 men and 7 women) The difference from the ex pected number did lie in the predicted direction but was not significant.

It should be noted that the mortality for both th female and male population of the whole country as in lose agreement with that for the towns lone For the male population there w some degree of exces mortality for the towns, especially in the ages over 60 years For the females on the other hand, there wa somewhat lower mortality If the mortality for the town population had been replaced by that for the population of the whole country the mortality observed in the asthma series would be practically the same

I 6 of these 15 cases (2 men nd 3 women) the deaths ere directly related to thmatic ttack Of the remaining ten patient one man with bronchiectasis nd history of chronic bronchitis died of renal nd circulatory insufficiency after an ttack of thma and broncho pneumonia which latter had been difficult to master clinically Four patients died of cerebral haemorrhage, cardiac infarct, diabetic gangrene nd cancer of the liver respectively One patient died post-operatively after bilateral thyroidectomy; the cause of death was considered t be cute telectasis, which affected both lungs simultaneously One

only when physical exercise was begun There appears to be some indication that the pressure increase is of a reversible nature, i e. it decreases or disappears when the disease becomes less active

The results of the investigation appeared to show that the orthostatic effect on the circulation in both the standing and the sitting positions as compared to the supine position was of a normal order of size in the asthma material

The stroke volume at rest in the asthma patients was on an average somewhat lower compared with normal control persons. On changing over from a resting state to physical work the asthma patients showed on an average a relatively great increase in the stroke volume. The arterio-venous oxygen difference in relation to oxygen uptake was on an average higher in the asthma series than in the control material — which mainly consisted of younger male persons. A hypokinetic" circulation was not judged to be present however as on an average the arterio-venous oxygen difference in relation to heart rate, as well as the increase of cardiac output in relation to increase of oxygen uptake during muscular work did not deviate from that of the control material. The asthma patients were therefore from this point of view regarded as "normokinetic

The relationship between pairs of different function variables was studied In the asthma material there appeared to be some relationship between several of these variables, and in some pairs this relationship was significant

The function variable which on being paired with others showed the lowest mean correlation was the ratio RV/TLC. In consideration of the relatively large increase in this ratio shown on an average by these patients a relationship with other variables would not have been unexpected. In this material no relationship was found between RV/TLC ratio and the pressure in the pulmonary artery or the pulmonary vascular resistance, not even when the two latter were calculated during physical exercise. This probably indicates that in patients with a high RV/TLC ratio hyperinflation complicated with alveolar tissue destruction occurred only to a small or moderate extent. It is considered possible that the reason may be found here for the fact that the hemodynamic findings were abnormal only to such a small extent in these patients. This fact is valid for the material as a whole but also to a certain extent for the group of patients in whom the severity of the asthma was assessed as pronounced. It was found, however that it was not always the values in patients with a history of particularly severe asthma that differed most greatly from the normal values of the function variables For this reason the patients who for certain function variables were found to show the greatest difference were studied separately This group of patients also exhibited a status that in several respects could be regarded as remarkably good considering that this material was composed of patients who during often great parts of their lives had been completely or partly disabled by asthma symptoms.

VIII SOCIO-MEDICAL ASPECTS

Mortality

Since in this investigation the cli nical course was not subjected to an independent and complete analysis, the mortality study was included in the socio-medical analysis and discussion. The mortality was studied both for the total asthma series and also for those patients who, on their first ad mission to hospital, were resident in the Uppsala urban district.

Mort ility study on the total asthma s ries i. e. all those patients who on admission to hospital fulfilled the ori ginal criteria for inclusion in the ma t rial

For a detailed significance analysis of the mortality the whole of the asthma series, i. e. 176 patients, were included. The calculations were made according to the principles described in Stati tical Methods (page 158). The expected number of deaths ac cording to th risk figures for Swedish towns was 5.31 for men and 5.00 for women (see Table 48). The risk figures for Swedish towns were used. This implies an approximation be cause only a few more than 80 / of th patients in the asthma series be-longed to the town population. The actual number of deaths among the 176 cases of the asthma series—i. e. all those who on admission to hospital fulfilled the criteria for inclusion in the material—was 15 (8 men and 7 women). The difference from the ex pected number did lie in the predicted direction but was not significant.

It hould be noted that the mortality for both the female and male population of the whole country w in less agreement with that for the towns lone. For the male population there was some degree of excess mortality for the towns, especially in the ages over 80 years. For the females, on the other hand, there wa somewhat lower mortality. If the mortality for the town population had been replaced by that for the population of the whole country the mortality observed in the asthma series would be practically the ame.

I 8 of these 15 cases (2 men and 3 women) the deaths were directly related to a asthmatic attack. Of the emaining ten patients one man with bronchiectasis nd a history of chronic bronchitis died f renal and circulatory n sufficiency after an ttack of asthma nd broncho-pneumonia which latter had been difficult to master clinically. Four patients died of cerebral haemorrhage, cardiac infarct, diabetic gangrene and cancer of the liver espectively. One patient died post-operatively after bilateral thyroidectomy; the cause of death was considered to be acute telectasis, which affected both lungs simultaneously. One

elderly man died at home—the cause of death was unknown. The cause of another patient's death was iatrogenic—not connected, however with the treatment of asthma. In two or possibly three cases the direct cause of death was considered to be broncho-pneumonia. In at least one of these cases it was believed that large doses of corticosteroids had contributed greatly not only to the occurrence of the pneumonia, but also to the fact that the latter was not mastered by the use of antibiotics and chemo therapy

Mortality study on those patients in the asthma series who on their first ad mission to hospital were resident in the Uppsala urban district

As a complement to the study of change of residence (see below) an in vestigation was made of the mortality of the urban district patients

Table 47 shows the survival proba bility figures for the 10 year period 1951—1960 (see mortality and life-ex pectancy tables in the official statistics for Sweden) for the Swedish town population in the respective age and sex groups Also shown in the table is the percentage of surviving male Upp sala town residents in the asthma series this figure was 88 5 (46/52) which may be compared with the corresponding figure for the Swedish towns which for a male population with the same age grouping as that of the asthma series was 92 4 The cor responding survival percentages for women were 92 3 (84/91) (asthma material) and 95 6 (the female town population) Thus the mortality ap peared to be higher in the asthma series but the difference was not sig nificant

---

To summarize it may be said that the observed mortality in the asthma series was somewhat higher than the expected This difference, however was not significant

Invalid pension

The studies made of the social situa tion of this group of asthma patients were based on information that was valid for the 1st November 1960

Of the 116 patients on whom the main proportion of the general ana lyses were based, 10 men and 11 wo-men were receiving an invalid pension at this date. The main reason in these patients was bronchial asthma. It was found that the 10 men received their pension at a mean age of 52 years (range 25—66 years) The correspon ding figure for the 11 women was 51 years (range 27—66 years)

Apart from these 21 patients who received an invalid pension, two women were also given an early pension by their employers o account of bronchial asthma In one case this was received at the age of 48 years, and in the other at 54 years. Since, however these two patients were not assessed in accordance with the norms of the National Social Insur nce Board, they were not incl ded as invalid pensioners in the analyses.

Of those who had other chronic d seases besides the bronchial asthma which were assessed as ha ing adverse influence n their life situation, their general condition and functions, six pati nts were recei i g in valid pension. Of these, three (two men, case Nos. 66 and 118, and one woman, No 43) were receiving a pensio because of bronchial a thma. Th oth r th ee (one man, No 112 and two women, N s. 77 and 101) w recei ing a pensi n on account of cerebr l throm bosis, pulmonary tuberculosis and cardia

arteriosclerosis, respectively. For the case histories of these patients reference may be made to page 160.

Change of residence

The frequency of change of residence was studied for those patients in the asthma series who on their first admission to hospital were resident in the urban district of Uppsala. A comparison was made with the total population of Uppsala.

The Uppsala urban population

The Uppsala urban population, and its distribution into sex and age, were known for the end of both 1950 and 1960. By a selection from the 1961 population schedule (which was valid for the end of 1960) the number of those who lived in Uppsala at the end of 1950 and were still resident there at the end of 1960 was estimated. Every 36th person was studied — one person on every page of the population schedule. The result of this selection study is shown in Table 45. By multiplying the number of persons selected by 36, an estimate of the total number of those who lived in Uppsala in 1950 and were still resident there in 1960 was obtained.

A calculation was then made of the percentage of the Uppsala urban population at the end of 1950 constituted by those still resident in Uppsala at the end of this ten year period (see Table 46, columns IV and V). The percentage of those still resident in the town can be estimated in two ways. The estimate shown in column IV Table 46, was obtained by multiplying the number still resident, according to the selection study by 36 (see Table 45, column IV and Table 46, column II) and dividing it by the number of persons resident in Uppsala in 1950 (see Table 46 column I). A better assessment is shown in column V Table 46. This takes into account the fact that the age and sex distribution in the selected persons was not identical with that shown by the 1960 population census (cf. columns III and V in Table 45). Firstly an estimate was made of the number of persons who were resident in Uppsala both in 1950 and 1960 (see Table 46, column III), then

Sex	Age group yrs.	Birth yr group	Selection: Resident in Uppsala at end of 1960 I	Selection: Of whom also resident in Uppsala at end of 1950 II	36 × selection: Resident in Uppsala at end of 1960 III	36 × selection: Of whom also resident in Uppsala at end of 1950 IV	Resident in Uppsala at end of 1960 acc. to population census V
Male	30—34	1926—1930	74	32	2664	1152	2724
	35—39	1921—1925	73	38	2628	1368	2601
	40—44	1916—1920	87	70	3132	2520	2513
	45—49	1911—1915	64	44	2304	1584	2197
	50—54	1906—1910	60	48	2160	1728	2226
	55—59	1901—1905	42	31	1512	1116	1836
	60—64	1896—1900	44	36	1584	1296	1620
	65—67	1893—1895	20	15	720	540	806
Female	30—34	1926—1930	67	31	2412	1116	2709
	35—39	1921—1925	71	43	2556	1548	2620
	40—44	1916—1920	85	57	3060	2052	2762
	45—49	1911—1915	88	63	3168	2268	2553
	50—54	1906—1910	78	53	2808	1908	2387
	55—59	1901—1905	62	47	2232	1692	2422
	60—64	1896—1900	55	37	1980	1332	2101
	65—67	1893—1895	28	19	1008	684	1289

Table 45. Result of selection study comprising every 36th person in the Uppsala population schedule for 1961 (referring to end of 1960).

$$\text{Column III in Table 46} = \frac{(\text{Column II in Table 45})}{(\text{Column I in Table 45})} \quad (\text{Column V in Table 45})$$

and then we obtain

$$\text{Column V in Table 46} = \frac{(\text{Column III in Table 46})}{(\text{Column I in Table 46})}$$

For the group of persons born in 1896—1900 for example, it was found that about 80 % of the men belonged to those who were still resident in Uppsala in 1960 while the corresponding figure for the women was 70 %. For the youngest age group of those born in 1926—1930 these figures were 51 % and 44 % respectively. As Table 40 shows, a great number had moved to other districts. It is evident that on the whole the percentage number of those still resident increased with increase in age.

The asthma series

The number of patients who lived in Uppsala at the time of their admission to hospital was 143 and for this group distributed according to age and sex, Table 47 gives the following information

1) the number who on admission to hospital fulfilled the criteria for inclusion in the material (column D)
2) the number who had died before the 1st November 1960 (column A)
3) the number out of those who were still alive on the 1st November 1960 who had moved to another district before this date (column B)
4) *the number who were still resident in Uppsala* on the 1st November 1960 (column C)
5) the mean year in which the patients were first admitted to hospital (column E)

Regarding the material as a whole, it was found that the number of men still resident in Uppsala on the 1st November 1960 was 30/52 and the number of women 73/91. The mean "first admission" year for the whole material was 1950.8, thus the mean observation period in this material was almost exactly ten years. By *first admission* is meant the first time a patient was ad

Year of birth	MALE: Resident in Uppsala 1950	MALE: Resident in Uppsala both 1950 and 1960		MALE: % still resident		FEMALE: Resident in Uppsala 1950	FEMALE: Resident in Uppsala both 1950 and 1960		FEMALE: % still resident	
				II/I	III/I				II/I	III/I
	I	II	III	IV	V	I	II	III	IV	V
1926—1930	2282	1162	1178	50.5	51.0	2837	1116	1233	39.4	41.2
1921—1925	2790	1368	1354	49.0	48.5	3126	1548	1708	49.5	54.0
1916—1920	2580	2520	2022	97.3	78.1	2803	2032	1832	[illegible]	[illegible]
1911—1915	2201	1584	1510	70.0	66.8	2309	2268	1837	00.4	[illegible]
1906—1910	2347	1728	1556	6.9	69.2	2450	1909	1 57	7 6	[illegible]
1901—1905	1832	1110	1353	60.0	74.0	2261	1602	1836	[illegible]	81.1
1896—1900	1644	1296	1325	78.8	80.6	1996	1332	1413	66.	70.8
1893—1895	1010	540	672	53.5	66.5	1220	684	820	56.1	67.2

Table 46. Estimated number and percentage of the 1950 population still resident in Uppsala 1960

The calculation of the percentage still resident was made in two ways (cf. Table 4). Column V shows the best estimate of the percentage still resident.
approximate number

mitted to hospital for asthma during the years 1945—1958 For many age groups, however this observation peri od was shorter or longer than ten years. The percentage number of those still resident was therefore corrected to correspond with an observation peri od of ten years (see column F) The correction formula used is given at the head of column F

Sex Year of birth	Asthma patients resident in Uppsala at first admission to hospital						Uppsala population still resident in Uppsala since 1948 acc. Col. V in Table	Expected percentage survivals during the 10-yr period 1951—60 for ... population	Difference F—G
	Died before 1.11.58	Alive 1.11.58 who moved to another district before 1.11.58	Still resident in Uppsala 1.11.58	Total A+B+C	First admission year (mean)	Percentage still resident corrected with regard to admission year acc. to the formula $1-\frac{10(D-C)}{D(1958.83-E)}$			
	A	B	C	D	E	F	G	H	
MALE									
1926—1930	0	1	5	6	1950.7	83.6	51.6	93.9	32.0
1921—1925	1	3	2	6	1948.5	43.9	48.5	96.6	— 3.6
1916—1920	0	2	2	4	1950.3	52.5	78.1	99.1	—25.6
1911—1915	0	0	5	5	1951.4	100.0	66.8	97.2	33.2
1906—1910	0	0	4	4	1952.8	100.0	69.2	95.5	30.8
1901—1905	1	0	7	8	1951.3	86.9	74.0	92.7	12.9
1896—1900	2	1	5	8	1952.0	57.5	80.0	88.8	—22.1
1891—1895	2	0	9	11	1952.6	77.0	66.3	82.0	11.4
Total Male	6	7	39	52	1951.2				
Standard weighted percentage figures (Col. D stand pop.)						73.0	67.2	92.4	7.8
FEMALE									
1926—1930*	0	3	6	9	1951.1	65.7	44.2	97.4	21.5
1921—1925	0	5	5	10	1945.9	48.1	51.0	99.1	3.6
1916—1920	2	0	4	6	1952.6	62.2	61.7	98.7	2.5
1911—1915	1	1	12	14	1950.5	86.2	72.8	99.0	13.4
1906—1910	0	0	6	6	1950.7	100.0	1	98.0	28.5
1901—1905	3	1	14	18	1950.7	78.0	81.1	95.2	— 3.1
1896—1900	0	0	17	17	1950.7	100.0	70.8	92.	29.2
1891—1895	1	1	9	11	1949.6	83.8	67.2	80.3	16.6
Total Female	7	11	73	91	1950.5				
Standard weighted percentage figures (Col. D stand pop.)						81.1	67.9	95.6	13.2

Table 47 Asthma patients who died, moved from and still lived in Uppsala respectively classified according to sex and age. The percentage still resident in Uppsala asthma patients and total Uppsala population. Expected mortality according to the mortality and life expectancy tables for the town population of Sweden for the 10-yr period 1951—60

* two women were born after 1930. They are combined in this and the following tables showing age distribution with the patients born in 1930.

$$\text{Column III in Table 46} = \frac{\text{(Column II in Table 45)}}{\text{(Column I in Table 45)}} \quad \text{(Column V in Table 45)}$$

and then, we obtain

$$\text{Column V in Table 46} = \frac{\text{(Column III in Table 46)}}{\text{(Column I in Table 46)}}$$

For the group of persons born in 1896—1900 for example, it was found that about 80 % of the men belonged to those who were still resident in Uppsala in 1960 while the corresponding figure for the women was 70 %. For the youngest age group of those born in 1926—1930 these figures were 51 % and 44 % respectively. As Table 46 shows a great number had moved to other districts. It is evident that on the whole the percentage number of those still resident increased with increase in age.

The asthma series

The number of patients who lived in Uppsala at the time of their admission to hospital was 143 and for this group distributed according to age and sex, Table 47 gives the following information

1) the number who on admission to hospital fulfilled the criteria for inclusion in the material (column D)
2) the number who had died before the 1st November 1960 (column A)
3) the number out of those who were still alive on the 1st November 1960 who had moved to another district before this date (column B)
4) the number who were still resident in Uppsala on the 1st November 1960 (column C)
5) the mean year in which the patients were first admitted to hospital (column E)

Regarding the material as a whole it was found that the number of men still resident in Uppsala on the 1st November 1960 was 30/52, and the number of women 73/91. The mean first admission year for the whole material was 1950.8, thus the mean observation period in this material was almost exactly ten years. By first admission is meant the first time a patient was ad

Year of birth	MALE: Resident in Uppsala 1950	MALE: Resident in Uppsala both 1950 and 1960		MALE: % still resident		FEMALE: Resident in Uppsala 1950	FEMALE: Resident in Uppsala both 1950 and 1960		FEMALE: % still resident	
				II/I	III/I				II/I	III/I
	I	II	III	IV	V	I	II	III	IV	V
1020—1030	2282	1152	1178	50.5	51.6	2832	1110	1253	39.4	44.2
1021—1925	2790	1368	1354	40.0	48.5	3126	1548	1708	49.5	54.6
1016—1920	2580	2520	2022	97.3	78.1	2863	2032	1852	1	64
1911—1015	2201	1584	1510	70.0	68.8	2500	2208	1827	00.4	73.8
1900—1910	2247	1728	1556	0.9	60.2	2459	1908	1757	7 6	1 3
1901—1905	1832	1116	1355	60.9	74.0	2264	1692	1836	4 7	81 1
1890—1900	1644	1296	1325	78.8	80.8	1908	1332	1413	66.	0 8
1803—1895	10[1]	540	072	53.5	66.5	1220[1]	681	820	56.1	6 2

Tabl 46. E ated number and percentage of the 1950 popul ti n still resident Uppsal 1960

The cal u on of th percentage still resident was mad in two ways (cf T ble 45) Col mn V sho e the best estimate of the percentage still resident.
approximat number

for the matriculation examination were 0.0 and 2, respectively. Even if the dif ference was not significant there was a tendency towards a greater number of examinations passed in the asthma series. On assessing these figures, how ever it must be taken into account that the control figures applied to the whole of Sweden, and the total popul ation in the collection area from which the patients in the asthma series were taken probably did not show agree ment with the total Swedish population in this respect. Uppsala — a long stand ing school and university town — may be assumed to show some over repre sentation with regard to both the lower certificate and the matriculation exa mination.

In order to find out how the patients in the asthma series compared with the total urban population of Uppsala, the material was selected in the same way as above but with the exclusion of the three patients from the rural districts. A study was made of the incidence of persons with an education comprising matriculation examination plus a uni versity degree or their equivalents. The expected number of such persons was calculated from the 1960 population census for the urban district of Uppsa la. Cons deration was taken of age and sex. The expected number with this education was found to be 1 7 and the observed number 2

T summari.e it may thus be said that from the point of view of edu cation the selected patients in the a thm series did not differ signifi cantly from the total population. Some tendency towards a greater number of ex min tions passed was observed in the asthma patients (see Table 48) The question of whether or not this tendency can be assumed to have any relationship to the disease will be discussed later

Family data

Marriage frequency The marriage frequency was estimated both in the whole main asthma series, i.e. those patients still alive and still resident in Uppsala on the 1st November 1960 (116 patients) and — in order to study the influence of the disease on this frequency — in a selection of 20 patients from this series, in whom the onset of asthma occurred at or before the age of 21 years and who up to the age of 30 years had had a maximum of two symptom free calen dar years. In both cases the expected number of persons who had at any time been married was calculated with the aid of information from the 1960 population census, consideration being taken of sex, age and district of resi dence i.e. whether the persons in question had li ed in the urban or the rural districts of Uppsala. The expec ted number of those who had at any time been married, in the whole main series, was 98.0 while the observed number was 101 The corresponding figures for the selected series were 24 6 and 24 respecti ely The patients in the asthma material did not thus appear to differ from the total popula tion in this respect (see Table 48)

Divorce The number of divorces was studied in the main asthma series, i.e those patients still alive and still resident in the collection area on the 1st November 1960 and who at any

The figures for those still resident in Uppsala on the 1st November 1960 appeared to be higher in the asthma series than in the Uppsala population. The age distribution of the asthma series was not the same as that of the Uppsala population. The older age groups were over represented in the asthma series and this age group in particular showed according to Table 46 the highest percentage figures for persons still resident in Uppsala at the given date Standard weighting was therefore performed, column D constituting the standard population.

Besides the data concerning the asthma series Table 47 shows in column G the percentage number of the 1950 Uppsala population still resident in this town on the 1st November 1960 (cf Table 46 column A)

It was found that the standard weighted percentage figures for those still resident in Uppsala was higher for the asthma series the difference for the men being 7 8 % for the women 13 2 % and for the whole series, regardless of sex, 11 2 % This latter difference is significant (**) The higher percentage figure for the asthma series is especially surprising since the series exhibited a somewhat higher mortality than the total population of the Swedish towns This emphasized the fact that the asthma patients showed a considerably lower tendency to move to other districts than the rest of the Uppsala population.

General education and family data

Adolesc ce and the early adult years c ltute the period when the basi both education and family formation is laid. Since in many of the patients in this series the age at the onset of asthma was relatively high, not all of the patients in the material were suitable for studies on the effect of the disease on these factors It seemed desirable, however to illustrate as far as possible the significance of the disease from the point of view of social adaptation Some questions of general education and family formation were therefore studied in a selected number of the more suitable patients from the series.

General education

In order to obtain some idea of the influence of this disease on the general education of the patients, a selection was made of those patients in the series in whom the asthma was first manifested at or before the age of 16 years and who between the ages of 16 and 24 years had had a maximum of two symptom free calendar years. These criteria were fulfilled by 22 patients, 11 of each sex. 19 of the 22 lived in the urban district of Uppsala, and the other three in the rural districts. The expected number of persons passing the lower certificate and matriculation examinations was calculated from the Historical Statistics of Sweden Consideration was taken of sex age and the annual number of examinations passed in the whole country during the period when these patients were of the ages 15—19 years and the number of persons of this age group in Sweden at the time in question The expected number of lower certificate examinations passed was 1.3 and the observed number 4 The corresponding figures

for the matriculation examination were 0.9 and 2 respectively. Even if the dif ference was not significant, there was a tendency towards a greater number of examinations passed in the asthma series. On assessing these figures, how ever it must be taken into account that the control figures applied to the whole of Sweden, and the total popul ation in the collection area from which the patients in the asthma series were taken probably did not show agreement with the total Swedish population in this respect. Uppsala — a long stand ing school and university town — may be assumed to show some over representation with regard to both the lower certificate and the matriculation examination.

In order to find out how the patients in the asthma series compared with the total urban population of Uppsala, the material was selected in the sam way as above but with the exclusion of the three patients from the rural districts. A study was made of the incidence of persons with an education comprising matriculation examination plus a uni ersity degree or their equivalents. The expected number of such persons was calculated from the 1960 population census for the urban district of Uppsa la. Consideration was taken of age and sex. The xpected number with this education was found to be 1 7 and the observed number 2.

T ummarize it may thus be said that from the point of view of edu cation the selected patients in the asthma series did not differ significantly from the total population. Some tendency towards a greater number of examinations passed was observed in the asthma patients (see Table 48). The question of whether or not this tendency can be assumed to have any relationship to the disease will be discussed later.

Family data

Marriage frequency. The marriage frequency was estimated both in the whole main asthma series, i. e. those patients still alive and still resident in Uppsala on the 1st November 1960 (116 patients) and — in order to study the influence of the disease on this frequency — in a selection of 29 patients from this series, in whom the onset of asthma occurred at or before the age of 21 years and who up to the age of 30 years had had a maximum of two symptom free calendar years. In both cases the expected number of persons who had at any time been married was calculated with the aid of information from the 1960 population census, consideration being taken of sex, age and district of resi dence, i. e. whether the persons in question had lived in the urban or the rural districts of Uppsala. The expec ted number of those who had at any time been married, in the whole main series, was 98.0 while the observed number was 101. The corresponding figures for the selected series were 24.6 and 24, respectively. The patients in the asthma material did not thus appear to differ from the total population in this respect (see Table 48).

Divorce. The number of divorces was studied in the main asthma series, i. e. those patients still alive and still resident in the collection area on the 1st November 1960 and who at any

The figures for those still resident in Uppsala on the 1st November 1960 appeared to be higher in the asthma series than in the Uppsala population. The age distribution of the asthma series was not the same as that of the Uppsala population The older age groups were over represented in the asthma series and this age group in particular showed according to Table 46 the highest percentage figures for persons still resident in Uppsala at the given date. Standard weighting was therefore performed, column D constituting the standard population.

Besides the data concerning the asthma series Table 47 shows, in column G the percentage number of the 1950 Uppsala population still resident in this town on the 1st November 1960 (cf Table 46 column V)

It was found that the standard weighted percentage figures for those still resident in Uppsala was higher for the asthma series the difference for the men being 7 8 % for the women 13 2 % and for the whole series, regardless of sex, 11 2 % This latter difference is significant (**) The higher percentage figure for the asthma series is especially surprising since the series exhibited a somewhat higher mortality than the total population of the Swedish towns. This emphasized the fact that the asthma patients showed a considerably lower tendency to move to other districts than the rest of the Uppsala population

General education and family data

Adolescence and the early adult years constitute the period when the basis of both education and family formation is laid. Since in many of the patients in this series the age at the onset of asthma was relatively high, not all of the patients in the material were suitable for studies on the effect of the disease on these factors. It seemed desirable, however to illustrate as far as possible the significance of the disease from the point of view of social adaptation. Some questions of general education and family formation were therefore studied in a selected number of the more suitable patients from the series.

General education

In order to obtain some idea of the influence of this disease on the general education of the patients, a selection was made of those patients in the series in whom the asthma was first manifested at or before the age of 16 years and who between the ages of 16 and 24 years had had a maximum of two symptom free calendar years. These criteria were fulfilled by 22 patients, 11 of each sex. 19 of the 22 lived in the urban district of Uppsala and the other three in the rural districts. The expected number of persons passing the lower certificate and matriculation examinations was calculated from the Historical Statistics of Sweden Consideration was taken of sex, age and the annual number of examinations passed in the whole country during the period when these patients were of the ages 15—19 years, and the number of persons of this age group in Sweden at the time in question. The expected number of lower certificate examinations passed was 1.3 and the observed number 4 The corresponding figures

Factor studied	Category of patients studied in the asthma material	N	Population from which the risk figures were taken for calculating the expected values	In calculating the expected values consideration was taken of	Observed No. in the asthma material	Expected figure
Dead	total series	1 6	the population of Sweden	age sex	15	10.31
	all men of total series	71	— —	—	8	4.31
	all women in total series	105	— —	— —	7	6 00
Still resident in Uppsala	all who on first admission to hospital were resident in Uppsala	143	Uppsala urban population 1950 and 1960	age in 5-yr groups sex	117*	96.1
Lower certificate examination	asthma onset at or before 16 yrs; maximum of 5 symptom-free yrs during the age 16—21	25	population of Sweden	No. of lower certificate examinations passed during the period when the patients in question were 15—19 yrs. and the No. of individuals in Sweden in this age group at the time in question, sex	4	1.3
Matriculation examination	— —	22	—	— —	2	0.8
Education requiring matriculation + university degree	resident in Uppsala; onset of asthma at or before age of 16 yrs; maximum of 2 symptom-free yrs during the ages 16—21	19	Uppsala urban population 1960	age in 5 or 10-y groups, sex	2	1.7
Married + previously married	total ... included in the main material	116	Uppsala urban and rural population 1960	age in ... groups	101	88
	those of this total whose asthma began at or before the age of 21 and who during the age of 20—30 had maximum of 2 symptom-free yrs	29	— —	— —	21	24.6
Divorced + previously divorced	total ... time married in the main material	101	Uppsala urban and rural population 1960	age in ... group	4	10.8
No. of children under 16 yrs living at home	married living together whose asthma began at or before 21 yrs. and who during the age 20—29 yrs. had maximum of 2 symptom-free yrs ... born 1924 or later and ... born 1921 or later	11	Uppsala urban and rural population 1960	age in 5 or 10-y groups women grouped to groups according to husband's age	23	17.2
	those of the above who are men	5	— —	— —	10	8.7
	those of the above who are women	6	—	— —	13	8.5

Table 18. Result of calculations of mortality, residence in Uppsala, school education, number married and divorced and the number of children under the age of 16 yrs. in patients in the asthma series. The Table shows the respective comparative populations and also the factors taken into account in calculating the expected figures. The degree of significance is ...

time had been married (101 patients) The expected number of those who in 1960 lived as divorced persons, was calculated with the aid of information from the 1960 population census, con sideration being taken of sex age and district of residence The expected number of divorced persons was 5.5 and the observed number 2 The dif ference is significant (*)

Apart from the two patients in the asthma series who in 1960 lived as divorced persons, a further two in this series had also at one time been divor ced. In order to find out how great a proportion of the married inhabitants of Uppsala had previously been divor ced, 1 558 persons of the age groups in question were selected at random from the records of the registrar s office. For each of these persons a note was made of whether or not they had previously been divorced. The pro-bands were divided into sex and age groups On the basis of this informa tion the expected number of previously divorced — but later re-married — persons was calculated for the asthma series This figure was 5 0 The diffe rence between the expected and ob-served figures was not significant. The difference might have increased some what however if the patients living in the rural district had also been in cluded since none of these had been divorced.

Thus the number of patients in the asthma series who were living as divorced or had previously been di orced but had later re married was 4 ompared with an expected figure of 0 5 This difference is significant (*) (see Table 48)

Number of children under the age of 16 years To obtain some idea of the fertility in the asthma material a study was made of the number of children under the age of 16 years in 1960 of married parents from the asthma series living with their husbands or wives respectively and in whom the asthma was first manifested at or before the age of 21 years and also who had had a maximum of two symptom free calen dar years between the ages of 21 and 30 years. Women born in 1925 or earlier and men born in 1920 or earlier were not included the information on the number of children for these pa tients could not be assessed since it was more probable that they could also have had children at or above the age of 16 years the official statistics only provide information for children under 16 years. The expected number of children was calculated from the 1960 population census consideration being taken of age and district of residence In this calculation of the expected number of children the women were counted as being of the same age as their respective husbands. The expec ted number of children for these pa tients regardless of sex was 17.3 and the observed number 23 The corres-ponding figures for the men were 8.7 and 10 respectively and for the women 8 5 and 13 respectively (see Table 48) The difference was not significant but supported the view that the patients in the asthma material did not have fewer children than the total popula tion

To summari e these patients did not appear to differ from the total popula tion with regard to marriage frequency

Factor studied	Category f patient studied in the thma material	No.	Populatio from which the ri k figures were taken f calculating the expected lues	I calcul ting th expected lues consideratio a taken f	Observed No. f these in the m terial	E pected figure
Died	total series	176	rla populatio of S eden	age sex	15	10.31
	all men i total series	71	— —	— —	8	5.31
	all women in total series	105	— —	—	7	5.00
Still resident in Uppsala	all who on first admis ion to hosp-ital were resident in Uppsala	143	Uppsala urba populatio 19.0 nd 1960	ge in 5-y groups, sex	112*	94.1
Lower cert ficate examination	asthma onset t or before 16 yrs maximum of 2 ymptom free rs. during the ge 16—21	22	population of S eden	No. of lower cert ficate examination pa sed during the period when the patient in question ere 15—19 yrs. nd the N of ind idual in weden in this age group at the time in question, sex	4	1.3
Matriculation examination	— —	22	— —	—	2	0.9
Education requiring matriculation + un ersity degree	resident Uppsala onset of sthma t or before ge of 16 yrs; maximum of 2 ymp tom-free yrs d ring the ages 16—21	19	Uppsala urban population 1960	age in 5 or 10 groups, sex	2	1 7
Married + previously married	total bo are n-cluded in the ma material	118	Uppsala urba and rural population 1960	ge in 5-y groups	101	94
	those of this total whose sthma bega 1 bef re the age of 21 yrs and ho during the ge f 20—29 had maxi-mum of 2 symptom free yrs.	29	—		21	21.6
Divorced + previously d vorced	total t m time married in the main material	101	Uppsala rba and rural population 1960	ge in 5- groups	4	10.5
No. of ch dren under 16 yrs living t home	married living together whose sthma bega t or befor 21 yrs. nd who during the age 20—29 yrs had ma imum of 2 symptom free yrs omen born 1921 later nd men born 1921 or later	11	Uppsala urba nd rural population 1960	ge 5 or 10-y groups men uped to groups ccording to husband' age	23	17.2
	those of the abov bo re men	5	— —	— —	10	8 7
	those of the above who are women	6	— —	— —	13	8.5

Table 45. *Results f calculations f mortality residenc in Uppsala, school educat n, numbe married divorced and the number of hildren nder the age f 16 yrs. in patient in the material. The Tabl shows the expective comparative populations and also the factors calculating the pected figures. The degree f significanc f assessed*

and the number of children. There was a tendency to fewer divorces in the asthma series than in the total population the difference between the observed and expected figures was significant.

Wage-earning

On studying the consequences of asthma an attempt was made to obtain as much information as possible in order to elucidate the *effect of the disease on the social situation* of the patients. It was considered *of interest* to classify the patients according to their branch of industry and occupational position. An attempt was also made to find out how many had *changed their occupations or had dis*continued to earn an income by reason of *asthma and also the incidence of* income-earners among the married women living with their husbands.

Distribution according to branch of industry

Table 49 shows the distribution into different branches of industry of the economically active men and women in this series of cases, compared with the *general* population of the same *district* according to the population census 1960 The figures given apply to the 1st November 1960

Economically active persons are — according to the population census 1960 — defined as those who during the so-called population *census week, carried out income-* [illegible] *ding* work comprising at least half the normal working hours. Also regarded as economically active were those persons who had income-yielding work but because of temporary discontinuation — *e g. vacation, illness, military repetition* exercises, temporary unemployment etc. — were not working during the population census week. The discontinuation was regarded as temporary if the duration of the interval was *not* more than 4 months The same criteria applied essentially to the patients in the asthma series.

The total number of inhabitants in the collection area was about 95,000 The number of persons who were born during the period 1893—1932 was approximately 44,000. Of these 81 9 [illegible] lived in the urban and 18.1 [illegible] in the rural district. The corresponding figures for the asthma series were 81 1 [illegible] and 18.9 *respectively*

Branch of Industry	Male			*Female*		
	Asthma series 1960		General population in the district of the asthma series 1960	Asthma series 1960		General population in the district of the asthma series 1960
	No	%	%	No	%	%
Agriculture, forestry work etc	6	19 4	12.3			2
Manufa[illegible]ring and handicraft trade	8	25 8	31.3	4	17 4	15 3
Buildi[illegible] trades	3	9 7	15.6			1 1
Com[illegible]	3	6 5	11 7	3	13 0	10 3
Tr[illegible] and communication	2	6.5	6.9	1	4 3	2 1
G[illegible] administration	10	32.3	22 0	15	65.[illegible]	59 1

T[illegible] 49 Economically active males and females in the asthma serie classified [illegible] to branch [illegible] industry compared with the corresponding figures for the general popul[illegible] in the same district 1960

In calculating the percentage figures of the control material consideration was taken of sex and district of residence but not of age, since there is no noteworthy relationship between industrial branch and age in the district concerned here. As the table shows, the distribution of the women between the different branches of industry was on the whole the same in the asthma series as in the total population. In the male asthma patients there was an over representation (non significant) in the group of general administration, some degree of over representation (non significant) in the category of agriculture and forestry work, and some degree of under representation (non-significant) in the groups of manufacturing handicraft and building trades, and commerce.

Distribution according to occupational status

For the 54 economically active patients the distribution between the two occupational positions employer and employed was studied. Of these 54 (31 men and 23 women) 42 were resident in the urban district of Uppsala and 12 in the rural districts. For all 54 the expected number of employers was calculated from information from the Uppsala 1960 population census. Consideration was taken of sex and age. It was found that in the asthma series there were 11 employers compared with the expected 4.8 This difference between the observed and expected figure was significant ( ) There was no noteworthy sex difference. When only those patients in the asthma series who lived in the urban district of Uppsala were studied, the observed number of employers was 8 while the expected number was 3.6. In this case the difference between the observed and expected figure did not reach the significance level.

Changes of occupation

Table 50 shows the changes of occupation among the patients in the asthma series. Only first changes were considered in this analysis. 30 % of the men stated that they had had to change their occupation on account of asthma, while the corresponding figure for the women was 11 %. This figure applied to the 30 women who in 1960 or earlier had income yielding work comprising at least half the normal hours (25 women had such work in 1960 and 11 women had previously had such work)

The mean age at the first change of occupation was 38 years for the men and 44 years for the women. On comparing the number of economically active persons in the different branches of industry before and after the change of occupation, it was found that 6 of the 7 men who had previously earned their living by agriculture and forestry work had left this branch of industry. The greatest inflow to any branch was found in the group of general administration. Even if tendencies such as these might be expected in view of the general interchanges between the different branches of industry during the last few decades, those observed in the asthma series seemed to be especially marked. There was an obvious ten

and the number of children There was a tendency to fewer *divorces in the* asthma series than in the total population the difference between the observed *and expected* figures was significant.

Wage-earning

On studying the consequences *of asthma* an attempt was made to obtain as much information as possible in order to elucidate the effect of the disease on the social situation *of the patients. It was considered of* interest to classify the patients according *to their branch of industry* and occupational position An attempt was also made to find *out how many had changed* their occupations or had discontinued to earn an income by reason of asthma and also the incidence of income-earners among the married women living with their husbands

Distribution according to branch of industry

Table 40 shows the distribution into different branches of industry of the economically active men and women *in this series of* cases, compared with the general population of the same district according to the population census 1960 The figures given apply to the 1st November 1960

Economically active persons are — according to the population census 1960 — defined as those who during the so-called population census week, carried out income yielding work comprising at least half the normal working hours. Also regarded as economically active were those persons who had income-yielding work *but because of temporary* discontinuation — e. g. vacation illness, military repetition exercises, temporary unemployment etc. — were not working during the population census week. The discontinuation was regarded a temporary if the duration of the interval was not more than 4 months. Th same criteria applied essentially to the patients in the asthma series

The total number of inhabitant in the collection area was about 95,000. The number of *persons who were born during* the period 1893—1932 was approximatel 45,000. Of these 81.9 / li ed in the urba and 18.1 / in the rural district The corresponding figures for the asthma series were 81 1 and 18.9 respectively

Branch of Industry	Male			Female		
	Asthma series 1960		General population i the district of the a thma s ries 1960	Asthma series 1960		General population the d s trict f the thma series 1960
	No	%	/	No	%	
Agriculture forestry work etc	6	19.4	12.2			2
Manufacturing and handicraft trade	8	26.8	31.2	4	17.4	16.3
B ilding trades	3	9.7	16.6			1.1
Commerce	2	6.5	11.7	3	13.0	10.3
Transport and communication	2	6.6	6.0	1	4.5	2.[illegible]
General admini tration	10	32.2	22.6	13	[illegible]	59.1

T bl 49. *Econ mically active males and femal s in the asthma eries classified according to branch of indu try compared with the corresponding f g res for the gen ral pop lati n the same district 1960*

ered that the change had had a f ourable effect on his sthma.

Case No. 13. L. E. Man, born 1925. Onset of asthma 1932. I 1930 the patient ga p orking as gardener because the flowers etc provoked symptoms of thma. H became re trained as fine-mechanic t a two-year course t an engineering college and had worked such ince 1932. He considered that the change of occupation had had f able effect on the course of the disease.

Case N 15. A. G. Woman, born 1921. Onset of sthma 1939. In 1935 she discontinued farm work, which had mainly consisted of tending cattle, nd underwent training to become children nurse as which she worked from 1933 own rds. She considered that the change f occupatio had had f our able effect on the course f her sthma.

Case No 1 H. E. Ma born 1923. Onset f sthma 1925 I 1917 he left f rm ork nd became orkshop labourer since several of the task hich he had to perf rm prov k ed asthma. H considered that the change had had f urable ffect on the course of the disease.

Case N 21. S. L. Man, born 1921. Onset of sthma 1930. In 1930 he hanged hi occupation from orkshop carpe ter to w lder bec use of the dust in his former place of work. He considered that the thma had improved since the change.

Case No 33 B. O. Ma born 1914. Onset of thma 1910. The patient had worked prev iously in tile factory but on account f the profuse dust he changed over in 1932 to wo k as plater. He considered that the change of occupation had ffected hi sthma fav urably.

Case No 40 J. N. Woman, born 1912. Onset f thma 1933. In 1931 the patient discontinued king shop assistant she f und this type of ork difficult on account f her sthma nd ince 1933 she had worked factory cleaner. She did not consider that the hange had had ny effect on the course of the asthma.

Case N 42 T. H. Man, born 1912. Onset of sthma 1938. Since the patient w unable to toler te the threshing dust among other things he hanged over from f rm ork to factory ork. H considered that the change had had fa ourable effect on the course of his asthma.

Case N 60 A. O. M n, born 1903. Onset of sthma 1911. I 1919 he discontinued hi work f mer since hi sthma wa worsened by much of the work that he had t perform. He hanged er to rious types f work the l y permanent position, but worked mostly salesma I 1931 he received in- lid pe ment lid f ne year. In 1939 he tried working in saw mill for short time but had to finish when he found that this made hi thma rse I 1939 he underwent a course t rehabilitation workshop after which he btained employment tores salesman, t which occupation he remained. The patient did not consider that his hanges of occupation had had y effect on the course f the sthma.

Case No. 61 L. E. W. man, born 190. Onset of sthma 1917 I 1926 she changed her occupation f om cook to hospital orderly since he wa unable to tolerate the smell of cooking. She did not consider that the change had had effect on the course of her thma.

Case No. 78 F. O. Man, born 1902. Onset of thma 192. In 1916 he changed er from f rmyard to forestry ork, since he w unable to tolerate the nimal H considered that the change had ffected his sthma fa ourably.

Case No 78 S. S. Man, born 1902. Onset of thma 1912. I alid pensio received since 1939. I that yea he partly disco tinued hi ork salesman, working only period which as report his asthma were mor fa ourable. H continued w th this work until 1947 when he changed er to part time ffice work, which he wa able to keep up for short periods off and on; the thma, however never permitted any permanent employment. The patient did not consider that the change of work had had any effect the course of the disease.

Case No 80 P. A. Man, born 1901. Onset of thma 1910. I 1938 he left his job in timber yard and btained work caretaker in block f flats. The reason for the change was partly that the former work invol ed considerable physical strain nd also that the dust affected his a thma. He considered that

dency to move from work involving physical strain to the more sedentary types of occupation. The demands of the different occupations on physical strain were assessed according to the norms established by Smårs and Berfenstam (1961) These authors distinguished between eight groups, in which the demand on physical strain decreased with increasing group numbers these were combined to form four groups in the present study Only 1 of the 15 men changed over to a heavier occupation while 8 changed to lighter and in 6 cases the working conditions were unchanged in this respect. The only employer before the change remained as such afterwards while one of the employees became an employer on changing his occupation.

The women included in this analysis were only few but the tendencies shown with regard to occupational changes appeared to agree with those of the men.

Some brief case-histories of those patients who changed their occupations by reason of asthma will be given below The descriptions include the year of onset of the asthma and the type of work performed by the patient before and after his change of occupation. Also mentioned are the factors in the original work that were considered to be unsuitable with regard to the asthma and the patient's personal opinion as to whether or not the change of work had had a favourable influence on the course of the disease.

Case N 4. A. A. Man, born 1929 Onset of a thma 1938. In 1950 he discontinued work as a salesman for a leather and skin firm, since the ski s provoked attacks f a thma Obtained employment as a metal worker after attending a re-training course. The patient considered that the change had had a fa ur able effect on the course of the disease

Case No 11 M J Man, born 1926. Onset of asthma 1942 Left farm work I 1945 and became a lorry dri er ince the contact w th cattle provoked asthmatic attacks. H con id

	Sex	No who changed	Average age at first change, yrs	Branch of industry: Agriculture forestry work etc	Manufacturing and handicraft trade	Building trades	Commerce	Transport and communication	General administration	No of employers	No of employees	Groups according to physical strain of the occupation: 1	2	3	4
Befo e first change	M	15	38	7	4	1	1	1	1	1	14	11	1	2	1
Aft				1	6	2	1	1	4	2	13	4	4	3	4
Befor first change	F	4	44	1	1		1		1		4		3	1	
Aft r					1				3		4			2	

ble 50 *Pat ents who changed their occupation becau e f bronchial asthma di tributed according to bran h of industry bef re and afte the fir t change f occupation*

roup according to physical strain of occupation based n those establi hed by Smårs and erfenstam (1961)

(7.5 %) of the men were included in both analyses, i. e. both in that of the changes of occupation and in that of the patients who had discontinued to earn an income. Of these three men there was one who after an interval of about two years returned to work.

The study with regard to the discontinuation of work showed — as in the case of changes of occupation — that several men gave up agricultural and forestry work. The same also applied to the manufacturing and handicraft trades, while the general administration branch lost relatively fewer workers. Of the men who had permanently topped working (10 altogether) one was an employer and the rest employees. There was a clear majority (8 out of 10) in the two groups in which the greatest degree of physical strain was demanded, and there were none in the group where the other extreme applied. The average age of the men at permanent discontinuation of income-earning work was 53 years.

With regard to the women, 10 (28 %) had permanently discontinued earning their living because of asthma. This percentage figure was based on the 36 women who at some time had had steady income-yielding work comprising at least 1/2 the usual working hours. The average age of the women at permanent discontinuation of income-earning work was 48 years.

Apart from these 10 women, a further four had discontinued earning an income but their work had comprised fewer than half time hours. The

	Sex	No	Average age at first discontinuation	Branch of industry which the patient left: Agriculture forestry work etc	Manufacturing and handicraft trade	Commerce	General administration	Before discontinuation: No of employers	No of employees	Groups according to physical strain in that occupation which the patient left: 1	2	3	4
Discontinued income-earning	M	10	53	3	4		3	1	9	4	4	2	
Discontinued income earning, but began again		2	40	1			1		2	1			1
Discontinued income-earning	F	10 (4)	48(57)	0(2)		3(0)	7(2)	1(1)	9(3)		2(2)	8(1)	0(1)
Discontinued income earning but began again		4	50		2		2		4		1	1	2

Table 61. *Discontinuation of income-earning because of asthma.*

These patients completely or by at least two-thirds discontinued income-earning, this being comprised at least half the usual working hours. The main reason for the discontinuation was bronchial asthma. The figures in parentheses refer to those whose occupation comprised fewer than half-time hours. The Table also shows those who after an interval of more than one year again began earning, with the same work as before or different, but this too comprising at least half the usual hours. Groups according to physical strain of occupation based on those established by Søndrn and Berfenstam (1961).

the course of the asthma had improved since he changed his occupation.

Case No 81 A. L. Man, born 1901 Onset of *asthma 1930. In 1950* he changed from pipe-laying to general labouring The reason was to avoid working in draughty places. The change was considered to have had no effect on the course of the asthma

Case No 103 V B Man, born 1897 Onset of asthma 1930 In 1945 he changed over from *driving a milk lorry to office work* — both positions at the Milk Distribution Centre. The reason for the change was that the patient was unable to manage the relatively heavy work as a lorry driver The change appeared to have no effect on the course of the asthma

Case No 113. E. W Woman, born 189. *Onset of asthma 1947 During the same year* she had to give up her work in a bakery (mainly as a bread packer) She was unable to work for almost a year because of severe asthma but then obtained employment in a brewery The patient's old place of employment was at that time unable to provid work for her and this was the main reason for the change She did not consider that the change of occupation had had any influence on the course of the disease

Case No 116. E. K Man, born 1895 Onset of asthma *1919 Recei ed an invalid pension* in 1951 on account of asthma In 1950 he gave up working as a combined farm labourer and builder and in the same year began part time work as an odd job man He consid red that the change had had a favourable effect on the course of the asthma since the *former work had in ol ed* too much physical strain.

Case N 125 H A. M n born 1893 Onset of a thma 1910 At th end of 1943 he left his work as a book binder and became a dle man fa tu er — *self-employed. He was unable* to t lerate the book dust in his old place f mployment. He considered that the a thma if anything had det ri rated since th change. He chose to change to the position of mployer mainly *because of his asthma*

One mal patient (case N 72) born 1 1903, whose asthma was first manifested in 194 was ex luded from T ble 50 Because his asthma was pro oked by contact with cattle h sold all the *cattle on his f rm in* 1952 and also part of the land, since the asthma had reduced his working capacity As, however the patient still maintained part of the farm and continued to earn a li ing as a farmer he was not included in the table The course of disease was *affected favourably* by the measures taken by the patient in 1952.

As shown by the case histories, if this latter patient was not included, 11 out of the 19 who had changed their occupations on account of asthma con sidered that this change had a favour able effect on the course of the disease. Seven considered that there was no effect, and one patient thought that his asthma had deteriorated somewhat by reason of his change of occupation.

Discontinuation of income yielding work

Table 51 shows the number of pa tients who mainly because of asthma had completely or by at least 2 3 per manently discontinued income yielding work which had comprised at least half the usual working hours The table also gives the number of patients who similarly discontinued work — *also of at least half the usual hours* — but began again after an interval of more than one year

As seen in the table 10 (24 %) of the men discontinued *permanently* while 2 (5 %) discontinued but after an interval of *more than one* year became economically active again Tak *ing into consideration the fact* that a further 15 (36 %) of the men in the *material found* it necessary to change their occupations, it is seen that a clear *majority* of the men were markedly affected by the disease in this respect It should be noted, however that 3

(7.5 %) of the men were included in both analyses, i. e. both in that of the changes of occupation and in that of the patients who had discontinued to earn an income. Of these three men there was one who after an interval of about two years returned to work.

The study with regard to the discontinuation of work showed — as in the case of changes of occupation — that several men gave up agricultural and forestry work. The same also applied to th manufacturing and handicraft trades, while the general administration branch lost relatively fewer workers. Of the men who had permanently stopped working (10 altogether) one was an employer and the rest employees. There was a clear majority (8 out of 10) in the two groups in which the greatest degree of physical strain was demanded and there were none in the group where the other extreme applied. The average age of the men at permanent discontinuation of income-earning work was 53 years.

With regard to the women, 10 (28 %) had permanently discontinued earning their living because of asthma. This percentage figure was based on the 36 women who at some time had had steady income-yielding work comprising at least ½ the usual working hours. The average age of the women at permanent discontinuation of income-earning work wa 48 years.

Apart from these 10 women a further four had discontinued earning an income but their work had comprised fewer than half time hours. The

	Sex	N	Average age at perm. discontinuation	Agriculture, forestry work etc.	Manufacturing and handicraft trade	Commerce	General administration	No. of employers	No. of employees	Groups according to physical strain in that occupation which the patient left: [illegible]	2	3	4
				Branch of industry which the patient left				Before discontinuation					
Discontinued income earning	M	10	53	3	6		3	1	9	4	4	2	
Discontinued income earning, but began again		3	40	1			1		2	1			1
Discontinued income-earning	F	10(14)	48(47)	0(3)		3(0)	7(2)	1(1)	9(3)		2(2)	8(1)	0(1)
Discontinued income-earning but began again		4	50		2		2		4		5	3	2

Table 41. *Discontinuation of income-earning because of asthma.*

These patients completely or by at least two-third discontinued income-earning, this having comprised at least half the usual working hours. The main reason for the discontinuation bronchial asthma. The figures in parenthesis refer to those whose occupation comprised fewer than half-time hours. The Table also shows those who after an interval of more than one year again began earning with the same work before or different, but this too comprised only half the usual hours. Groups according to physical strain of occupation based on those established by Sellers and Berkowitz (1951).

figures given in parenthesis in the table apply to theese women. They are not included in the ordinary figures.

The table also shows that four other women had similarly ceased working because of bronchial asthma but they had later returned to the same or a different occupation after an interval of more than one year.

The table includes (in parenthesis) two farmers' wives who stated that the family farming property had had to be sold for the main reason that the wives were unable to take part in the outdoor work because of asthma. In one of these cases the husband was a semi invalid and in both cases the husbands and wives were about 60 years old. It was rather difficult, however, to determine the predominant reason for the disposal of the farms. These women are included in the table among those whose work was assessed as comprising fewer than half of the usual working hours.

Income yielding work among married women living with their husbands

Table 52 shows the number of economically active and inactive married women living with their husbands, in both the asthma series and the Uppsala population, distributed according to age. As may be seen in the table, there is almost exact agreement between the two entire series with regard to the incidence of persons with income yielding work. In two of the different age groups there is a somewhat greater difference between the asthma series and the Uppsala population but regarded separately these two groups are much too small to permit any true assessment. The figures for the Uppsala population were calculated from the population census of the 1st November 1960.

To summarize it may be said that the distribution of the economically active patients in the asthma material into different branches of industry did not differ significantly from that in the total population. A study of the

Age, years	30—39			40—49			50—59			60—69			70—79		
	Asthma series		Corresponding general population of Uppsala	Asthma series		Corresponding general population of Uppsala	Asthma series		Corresponding general population of Uppsala	Asthma series		Corresponding general population of Uppsala	Asthma series		Corresponding general population of Uppsala
	No.	%	%	No.	%	%	No.	%	%	No.	%	%	No.	%	%
Economically active	2	22.2	32.2	8	61.5	36.0	4	30.8	28.2	1	6.3	8.9	1	29.4	29.0
Economically inactive	7	77.8	67.8	5	38.5	64.0	9	69.2	71.8	15	93	91.1	30	70.6	71.0
Total	9	100.0	100.0	13	100.0	100.0	13	100.0	100.0	16	100.0	100.0	1	100.0	100.0

Table 52. *Economically active and inactive non invalid pensioned women in the asthma series married and living with their husbands distributed by age compared with the corresponding figures for the general population of Uppsala 1960.*

By economically active women is meant here those with income-yielding work comprising at least half the ordinary working hours.

distribution into occupational positions showed that a relatively great number of the asthma patients in relation to the Uppsala population were to be found in the group of self-employers.

About every 3rd man and every 10th woman changed their occupation on at least one occasion on account of asthma. Approximately every 4th man and woman discontinued income-yielding work permanently because of asthma. No significant difference compared with the Uppsala population was shown with regard to the incidence of persons with income-yielding work among the married women in the asthma series, living with their husbands.

Investigation of the income level of the economically active patients

A study was made of the income level in December 1963 of the non-invalid pensioned patients born in 1897 or later using as a basis the sickness benefit classes to which they were assigned by the General Sickness Insurance. 1963 was chosen because this was the first year in which not only income earned as an employee but also income obtained by different forms of self-employment was taken into consideration at the General Sickness Insurance, where there is a relationship between income level and the sickness benefit class to which a person is assigned. Corresponding to each of the sickness benefit classes there is a certain amount of daily sickness benefit to which a person is entitled. The amount of this sickness benefit in different income-level groups is shown in Table 53. The table also shows that the income scale is only differentiated up to 21 000 Swedish crowns of annual income.

Information was obtained as to the amount of sickness benefit to which each of the patients in the asthma series was entitled. For control purposes information was also obtained with regard to the corresponding amount of sickness benefit for the two persons — of the same sex as the patient — entered in the register of the General Sickness Insurance immediately before and the two persons entered immediately after the asthma patient concerned. This register is made up according to the date of birth of the insured person. The sickness benefit of these four persons were added together and the figure obtained divided by four. The final figure was then compared with the benefit to which the asthma patient was entitled. For each asthma patient his own figure and a control figure were thus obtained. Since invalid pensioners were excluded from the asthma material they were also excluded from this control material.

By the procedure described above it was possible to obtain a mean group figure both for the men and for the women of the asthma and the control materials. For the men in the asthma

Annual earned income		Sickness benefit
reached Sw. Cr.	but not Sw. Cr.	Sw. Cr.
1800	2200	5
2200	3400	6
3400	4200	7
4200	5000	8
5000	5800	9
5800	6800	10
6800	8400	12
8400	10200	14
10200	12000	16
12000	14000	18
14000	16000	20
16000	18000	22
18000	21000	25
21000	—	28

Table 53. The daily sickness benefit from the General Sickness Insurance in different income levels.

figures given in parenthesis in the table apply to theese women They are not included in the ordinary figures.

The table also shows that four other women had similary ceased working because of bronchial asthma but they had later returned to the same or a different occupation after an interval of more than one year

The table includes (in parenthesis) two farmers wives who stated that the family farming property had had to be sold for the main reason that the wi es were unable to take part in the outdoor work because of asthma In one of these cases the husband was a semi invalid and in both cases the husband and wives were about 60 years old. It was rather difficult, however to determine the predominant reason for the disposal of the farms. These wom n are included in the table among those whose work was assessed as comprising fewer than half of the usual work ing hours.

Income yielding work among married women living with their husbands

Table 52 shows the number of economically active and inactive married women, living with their husbands, in both the asthma series and the Uppsala population distributed according to age. As may be seen in the table there is almost exact agreement between the two entire series with regard to the in cidence of persons with income-yielding work. In two of the different age groups there is a somewhat greater difference between the asthma series and the Uppsala population, but regarded separately these two groups are much too small to permit any true as sessment The figures for the Uppsala population were calculated from the population census of the 1st November 1960

To summari e it may be said that the distribution of the economically active patients in the asthma material into different branches of industry did not differ significantly from that in the total population. A study of the

Age years	30—39			40—49			50—59			60—69			30—69		
	Asthma series		Corresponding general popula tion f Uppsala	Asthma-series		Corresponding general popula tion f Uppsala	Asthma-series		Corresponding general popula tion f Uppsala	Asthma-series		Corresponding general popula tion f Uppsala	Asthma series		Corresponding general popula tion f Uppsala
	No	%	%	No.	%	%	No.	%	%	No	%	%	No.	%	%
Econ mic lly acti		22.2	33.2	8	61.5	36.0	4	30.8	38.2	1	6 3	8.9	13	20 4	20 0
Econom call ina ll		B	67.8	-	38.5	64.0	0	60 2	1.8	1	93	91.1	30	70 6	1.0
Total	0	100 0	100 0	13	100 0	100.0	13	100.0	100 0	10	100.0	100 0	31	100 0	100.0

Table 5_ Economically active and inactive n n invalid pensi ned women in the asthma seri married and living with their hu bands, distributed by age compared with th corre ponding figure f the general popul ti n of Uppsal 1960

By economically active women" is meant here those with income- ieldi g work compri i g t les t half the ordinary w rking hours.

distribution into occupational positions showed that a relatively great number of the asthma patients in relation to the Uppsala population were to be found in the group of self-employers.

About every 3rd man and every *10th woman changed their occupation* on at least one occasion on account of asthma. Approximately every 4th man and woman discontinued income-yielding work permanently because of asthma. No significant difference compared with the Uppsala population was shown with regard to the incidence of persons with income-yielding work among the married women in the asthma series, living with their husbands.

Investigation of the income level of the economically active patients

A study was made of the income level in December 1963 of the non-invalid pensioned patients born in 1897 or later using as a basis the sickness benefit classes to which they were assigned by the General Sickness Insurance. 1963 was chosen because this was the first year in which not only income earned as an employee but also income obtained by different forms of self employment was taken into consideration at the General Sickness Insurance, where there is a relationship between income level and the sickness benefit class to which a person is assigned. Corresponding to each of the sickness benefit classes there is a certain amount of daily sickness benefit to which a person is entitled. The amount of this sickness benefit in different income-level groups is shown in *Table 33*. The table also shows that the income scale is only differentiated up to 21 000 Swedish crowns of annual income.

Information was obtained as to the amount of sickness benefit to which each of the patients in the asthma series was entitled. For control purposes information was also obtained with regard to the corresponding amount of sickness benefit for the two persons of the same sex as the patient entered in the register of the General Sickness Insurance immediately before and the two persons entered immediately after the asthma patient concerned. This register is made up according to the date of birth of the insured person. The sickness benefit for these four persons were added together and the figure obtained divided by four. The final figure was then compared with the benefit to which the asthma patient was entitled. For each asthma patient his own figure and a control figure were thus obtained. Since invalid pensioners were excluded from the asthma material they were also excluded from this control material.

By the procedure described above it was possible to obtain a mean group figure both for the men and for the women of the asthma and the control materials. For the men in the asthma

Annual earned income		Sickness benefit
exceeded Sw. Cr	but not Sw. Cr	Sw. Cr
1800	2000	5
2000	2400	6
2400	3200	7
3200	4000	8
4000	5400	9
5400	6800	10
6800	8400	12
8400	10000	14
10000	12000	16
12000	14000	18
14000	16000	20
16000	18000	22
18000	21000	24
21000	—	26

Table 33. The daily sickness benefit from the General Sickness Insurance in different income levels

figures given in parenthesis in the table apply to theese women They are not included in the ordinary figures

The table also shows that four other women had similary ceased working because of bronchial asthma but they had later returned to the same or a different occupation after an interval of more than one year

The talle includes (in parenthesis) two farmers wives who stated that the family farming property had had to be sold for the main reason that the wives were unable to take part in the outdoor work because of asthma In one of these cases the husband was a semi invalid and in both cases the husbands and wives were about 00 years old It was rather difficult, however to determine the predominant reason for the disposal of the farms. These women are included in the table among those whose work was assessed as comprising few r than half of the usual working hours.

Income yielding work among married women living with their husbands

Table 52 shows the number of economically active and inactive married women, living with their husbands, in both the asthma series and the Uppsala population distributed according to age. As may be seen in the table, there is almost exact agreement between the two entire series with regard to the incidence of persons with income yield ing work. In two of the different age groups there is a somewhat greater difference between the asthma series and the Uppsala population but regarded separately these two groups are much too small to permit any true assessment. The figures for the Uppsala population were calculated from the population census of the 1st November 1960

To summari e it may be said that the distribution of the economically active patients in the asthma material into different branches of industry did not differ significantly from that in the total population. A study of the

Age years	30—39			40—49			50—59			60—69			30—69		
	Asthma series		Corresponding general population of Uppsala	Asthma series		Corresponding general popula tion f Uppsala	Asthma series		Corresponding general popula tion f Uppsala	Asthma-series		Corresponding general popula tion of Uppsala	Asthma series		Corresponding general popula tion f Uppsal
	No.	%	%	No.	%	%	No	%	%	No.	%	%	No.	%	%
Eco omically acti	2	22.2	32 2	8	61.5	36 0	4	30.8	28 2	1	6 3	8.9	15	30 4	29 0
Eco mically Inacti		77.8	67 8	5	38.5	61 0	9	69 2	71.8	15	93.7	91 1	30	70 6	1.0
Total	9	100 0	100.0	13	100 0	100.0	13	100.0	100 0	16	100.0	100 0	[illegible]	100 0	100 0

Table 5_ *Economically active and inactive n n invalid pensi ned women in the asthma eries married and living with their hu bands dist ibuted by age compared with the corresponding figure f the general population of Uppsal 1960*

By economically active wom n I mea t here those with income-yi lding work compri ing t les t half the ordinary wo king ho rs.

corresponding figures for December 1960 and December 1963 were 48 % and 50 % respectively. The tendency in these men in the asthma series towards an improvement in their level of income during the period studied, in comparison with the control material, did not reach a degree of significance.

Out of all the employed men in the asthma series in 1955 one was excluded from this study because he received an invalid pension after that year.

To summari~e the mean income of the men in the asthma series born in 189 or later and who were not receiv ing an invalid pension, did not differ significantly from the expected figure. The mean income of the economically active women in the asthma series, on the other hand, was lower than the ex pected figure. The incidence of economically active women in the asthma material was in close agreement with that in the total population.

The income development of the economically active employed men in the asthma series during the period 1955—63 did not appear to differ significantly from that of the total population.

Housing standard and size

Housi g standard

It is often said that there is a certain degree of relationship between poor housing standards and asthma. The study of the standard of housing of these patients was therefore of interest not only as a measure of their social adaptation but also in seeking to obtain an idea of the extent to which such a relationship might apply to the individuals in the asthma series. In an attempt to determine the degree to which the apartments of these patients could be said to fulfil reasonable de mands of quality and size the housing *standard of each patient was compared* with that of the so-called median per son living in the same district and belonging to the same civil state and age group.

In classifying the apartments from a point of view of standard, the prin ciples shown in Table 54 were used, the standard of the apartment decreasing with increasing group numbers. An apartment for example, filling all reasonal le requirements of modern standard, was assigned to group 1 while its opposite was placed in group 7. It was found that 70 % of the patients lived in apartments of stand ard degree 1 5 % of degree 2, 8 % of legree 3 6 /. of degree 4 1 % of degree 5 8 /. of degree 6 and .. % of degree 7

with water and drain						
with w and central heating			with w without central heating	witho t w.c.		
ith ow bathroom or shower		without wn bathroom or shower		with central heating	without central heating	without water and/or drain
ith modern cooker nd refrigerator	without modern cooker and/or refrigerator					
standard-gr 1	st-gr 2	st-gr 3	t-gr 4	t-gr 5	st-gr 6	st-gr 7

Table 54 *Classification of ho sing conditi ns into standard groups.*

series this was 19 9 Swedish crowns, and for the men in the control material 20 0 Swedish crowns 13 of the men in the asthma series attained a higher figure than the mean figure for the men in the control material with whom each of the patients was compared 13 attained a lower figure and 2 showed the same figure

In the asthma series there were 53 women who were born in 1897 or later and who were not receiving invalid pensions Of these 24 (45.3 %) were registered at the General Sickness Insurance as having an earned income exceeding 1800 Swedish crowns per year These patients were entitled to a mean daily sickness benefit payment of 12 0 Swedish crowns. As mentioned above for each of these 53 women there were four control persons. Of these 212 90 (42 5 %) were registered as having an earned income, and they were entitled to a mean daily sickness benefit payment of 15 2 Swedish crowns Thus the sickness benefit to which the women in the asthma series who were registered as having an earned income, were entitled was on an average lower than the corresponding benefit in the control material The difference between the asthma series and the control material was significant ( ) On the other hand there was no significant difference with regard to the percentage number of women with an estimated annual income of at least 1800 Swedish crowns

When the male self employers were excluded from both the asthma series and the control material a mean figure of 21 7 Swedish crowns was obtained for the asthma series, while the corresponding figure for the control material was 21 1 Swedish crowns. With regard to the men in the asthma series, nine attained a higher figure than the mean figure for the men in the control material with whom each of the patients were compared, ten showed a lower figure and two the same figure

To obtain an idea of the income development during the period 1955—1963 for the men in the asthma series born in 1897 or later who in 1963 were not receiving an invalid pension, and who were not self-employed during the period 1955—1963 the following method was used. (Before the year 1963 self employers were not registered by the General Sickness Insurance according to their income levels and could therefore not be included)

The men in the a thma serie were di ided into groups ccording to ge and the district in which they li ed and the men in the t tal I ppsala population were grouped in a s milar way Each man in the asthma series was then ranked as described on page 31 in relatio to the median person in the total population, each patient being given a percentage f gur which i dicated hi income level relation to that of the median pers n, which was determi ned as 50 %. This method was used for comparing the mean incom level i the th ma series and in the control material f December 1955 and f r December 1960 The procedure wa principally th sam when a comparison wa mad fo December 1963 Th patients in th thma series were divided into age groups. Each patient was then ranked in relatio t the control material f r December 1963, btained from the General Sickness Insurance still with the e l ion of the self-employed.

It was found that for December 1955 the men in the asthma series reached a mean ranking figure of 40 % The

corresponding figures for December 1960 and December 1963 were 40 % and 50 % respectively. The tendency in these men in the asthma series towards an improvement in their level of income during the period studied, in comparison with the control material, did not reach a degree of significance.

Out of all the employed men in the asthma series in 1955 one was excluded from this study because he received an invalid pension after that year.

To summari e the mean income of the men in the asthma series born in 1897 or later and who were not receiv ing an invalid pension, did not differ significantly from the expected figure. The mean income of the economically active women in the asthma series, on the other hand, was lower than the ex pected figure. The incidence of economically acti e women in the asthma material was in close agreement with that in the total population.

The income development of the economically acti e employed men in the asthma series during the period 1955—63 did not appear to differ significantly from that of the total population.

Housing standard and size

Housing standard

It is often said that there is a certain degree of relationship between poor housing standards and asthma. The study of the standard of housing of these patients was therefore of interest not only as a measure of their social adaptation but also in seeking to obtain an idea of the extent to which such a relationship might apply to the individuals in the asthma series. In an attempt to determine the degree to which the apartments of these patients could be said to fulfil reasonable de mands of quality and size the housing standard of each patient was compared with that of the so-called median per son living in the same district and belonging to the same civil state and age group.

In classifying the apartments from a point of view of standard, the prin ciples shown in Table 54 were used, the standard of the apartment decreasing with increasing group numbers. An apartment for example filling all reasonable requirements of modern standard, was assigned to group 1 while its opposite was placed in group 7. It was found that 70 % of the patients l ed in apartments of standard degree 1 5 % of degree 2, 8 % of degree 3 6 / of degree 4 1 % of degree 5, 6 / of degree 6 and 2 % of degree 7.

with w ter and drains						
with w and central heating			with w.c., w thout central heating	without w.c.		
with own bathroom or shower		w thout own bathroom or shower				
with modern cooker and refrigerator	without modern cooker and/or efrigerato			ith central heating	ithout central heating	without ter nd/or drain
standard-gr 1	st.-gr 2	st.-gr 3	t. gr 4	st.-gr 4	st.-gr 6	t-gr 7

T ble 54 *Classification / housing conditions int standard groups.*

series this was 19 9 Swedish crowns, and for the men in the control material 20 0 Swedish crowns. 13 of the men in the asthma series attained a higher figure than the mean figure for the men in the control material with whom each of the patients was compared 13 attained a lower figure and 2 showed the same figure

In the asthma series there were 53 women who were born in 1897 or later and who were not receiving invalid pensions Of these, 24 (45.3 %) were registered at the General Sickness Insurance as having an earned income exceeding 1800 Swedish crowns per year These patients were entitled to a mean daily sickness benefit payment of 12 0 Swedish crowns As mentioned above, for each of these 53 women there were four control persons Of these 212 90 (42.5 %) were registered as having an earned income, and they were entitled to a mean daily sickness benefit payment of 15 2 Swedish crowns. Thus the sickness benefit to which the women in the asthma series, who were registered as having an earned income, were entitled, was on an average lower than the corresponding benefit in the control material. The difference between the asthma series and the control material was significant (*) On the other hand there was no significant difference with regard to the percentage number of women with an estimated annual income of at least 1800 Swedish crowns

When the male self employers were excluded from both the asthma series and the control material, a mean figure of 21 7 Swedish crowns was obtained for the asthma series, while the corresponding figure for the control material was 21 1 Swedish crowns. With regard to the men in the asthma series, nine attained a higher figure than the mean figure for the men in the control material with whom each of the patients were compared, ten showed a lower figure and two the same figure.

To obtain an idea of the income development during the period 1955—1963 for the men in the asthma series born in 1897 or later who in 1963 were not receiving an invalid pension and who were not self employed during the period 1955—1963 the following method was used. (Before the year 1963 self-employers were not registered by the General Sickness Insurance according to their income levels and could therefore not be included)

The men in the asthma series were divided into groups according to age and the district in which they lived, and the men in the total Uppsala population were grouped in a similar way Each man in the asthma series was then ranked as described on page 31 in relation to the median person in the total population, each patient being given a percentage figure which indicated his income level in relation to that of the median person which was determined as 50 %. This method was used for comparing the mean income level in the asthma series and the control material for December 1955 and for December 1960 The procedure was principally the same when comparison was made for December 1963 The patients in the asthma series were divided into age groups Each patient was then ranked in relation to the control material for December 1963 obtained from the General Sickness Insurance, still with the exclusion of the self-employed.

It was found that for December 1955 the men in the asthma series reached a mean ranking figure of 40 % The

corresponding figures for December 1960 and December 1963 were 40 % and 50 % respectively. The tendency in these men in the asthma series towards an improvement in their level of income during the period studied, in comparison with the control material, did not reach a degree of significance.

Out of all the employed men in the asthma series in 1955 one was excluded from this study because he received an invalid pension after that year.

To summari e the mean income of the men in the asthma series born in 1897 or later and who were not receiving an invalid pension, did not differ significantly from the expected figure. The mean income of the economically active women in the asthma series, on the other hand, was lower than the expected figure. The incidence of economically active women in the asthma material was in close agreement with that in the total population.

The income development of the economically acti e employed men in the asthma series during the period 1955—63 did not appear to differ significantly from that of the total population.

Housing standard and size

Housing standard

It is often said that there is a certain degree of relationship between poor housing standards and asthma. The study of the standard of housing of these patients was therefore of interest not only as a measure of their social adaptation but also in seeking to obtain an idea of the extent to which such a relationship might apply to the individuals in the asthma series. In an attempt to determine the degree to which the apartments of these patients could be said to fulfil reasonable demands of quality and size the housing standard of each patient was compared with that of the so-called median person living in the same district and belonging to the same civil state and age group.

In classifying the apartments from a point of view of standard, the principles shown in Table 54 were used, the standard of the apartment decreasing with increasing group numbers. An apartment for example, filling all reasonable requirements of modern standard was assigned to group 1 whil its opposite was placed in group 7. It was found that 70 % of the patients lived in apartments of standard degree 1 5 % of degree 2 8 % of degree 3 0 % of degree 4 1 % of degree 5, 8 % of degree 6 and 2 % of degree 7.

with water and drain						
with w and central heating			with w.c., without central heating	without w.c.		
th own bathroom or shower		without own bathroom or shower		ith central heating	thout central heating	without w ter and/or drains
th modern cooker and refrigerator	without modern cooker and/or refrigerator					
Standard gr 1	al gr 2	L-gr 3	l-gr 4	aL-gr 5	L-gr 6	L-gr 7

Table 54 Classification of housing conditions int standard groups.

Table 55 shows the housing stand ard in the asthma series each patient being compared with the median per son in the total population according to the so-called "ranking method described on page 31 The table di stinguishes between heads of the house hold belonging to the asthma series i e men and unmarried or divorced women and widows and heads of the household who belong to the rest of the population i e. men who are mar ried and live with their wives who belong to the asthma series As seen in the table the asthma series as a whole compared well with the total Uppsala population. The median con trol person in the total population is placed at figure 50 None of the groups shown in the table differed significant ly from the expected figure of 50

As seen in Table 56 the mean ran king figure for the housing standard for the total asthma series was 53.6. Those living in the rural districts had a somewhat higher figure and those in the urban districts somewhat lower When the series was divided into two groups, one in which the patients themselves were heads of the house hold and one in which the patient s husband was the head of the house-hold a mean figure of 50 , was obtained for the former and 56.3 for the latter

For the invalid pensioners included in the series the mean figure was 52.4 and when these were divided into two groups according to whether or not the head of the household was part of the asthma series the figures obtained were 51 2 and 54 0 respectively The corres

Groups acc to age yrs.		Groups according to so-called ranking figures					Mean ranking figure	
		0 20	21—40	41—60	61—80	81—100		
30—34	I	2		2	2		43.6	44.8
	II	1		3			46	
35—39	I	1		2	2		5_0	54 4
	II			4			5 4	
40—44	I			1			58.1	61 1
	II			4	1		61 6	
45—49	I	3		2			40 3	48 0
	II			2	1		63 5	
50—54	I			1	4	1	66 3	58 3
	II	1	1		9		53.9	
55—64	I	2	5	2	13	1	52 5	55.0
	II	2	1	3	11	2	5 7	
65—	I	3	1		4		40 0	4 6
	II	2	2		10		51	

Table 55 *Standard of housing conditions 1960 according to age for each of the individual in the asthma series expressed in accord nce with th so-called ranking method (page 31) wher each individ ual in the asthma eries is compared with the so-called median per on within the same ag ex and ci il tate group in the total popul tion (urban and rural dist i t popul tions)*

I = hr i of household, included in the asthma ries.
II = h ad of h usehold, living with wife wh is included in the asthma series.

ponding figures for the non invalid pensioners in the series were 51.2 and 55.8 respectively.

When the patients in the total series were divided into groups according to the mean annual number of sickness points during the period 1957—61 it was found that those who during this period belonged to group 3, i.e. those with the most symptoms (estimated mean annual sickness points of 100 or more) had a mean ranking figure of 51.4. Within this group there was a not unessential difference between the group in which the head of the household was included in the asthma series which group showed a figure of 43.2, and that comprising married women living with their husbands where the husbands were head of the household. This latter category showed a figure of 64.2. For those on the other hand, who during the same period belonged to the group with the least symptoms accord

Category	No.	Ranking figure	
		Standard	Size
Whole mal asthma series	116	53.6	47.3
Town residents	94	52.9	45.1
Rural district residents	22	56.6	56.8
Unmarried single and other women who are themselves head of the household	56	50.7	46.3
Married women living with their husbands, where the husband is head of the household	60	56.3	49.3
Invalid pensioners, total	21	52.4	37.0
Invalid pensioners who are themselves head of the household	13	51.2	36.1
Invalid pensioners, whose husband are head of the household	8	54.0	37.7
Non invalid pensioners, total	95	53.8	49.6
Non invalid pensioners who are themselves head of the household	42	51.3	
Non invalid pensioners, whose husband are head of the household	53	53.8	
Patients who during the period 1957—61 attained an annual mean sickness point figure of 100 or more	18	51.4	39.3
Those of these patients who are themselves head of the household	11	43.2	
Those of these patients whose husband are head of the household	7	64.2	
Patients who during the period 1957—61 attained annual mean sickness point figure of less than 60	60	54.8	50.6
Those of these patients who are themselves head of the household	31	53.7	
Those of these patients, whose husbands are head of the household	28	53.7	
Patients who according to Table 3 belong to group A, i.e. up to the end of 1960 acquired fewer than 1000 sickness points	38	54.9	57.3
Those of these patients who are themselves head of the household	15	60.8	59.8
Those of these patients, whose husband are head of the household	23	51.3	53.6
Patients who according to Table 3 belong to group B, i.e. up to the end of 1960 acquired 1000 but not 2000 sickness points	41	54.4	42.7
Those of these patients who are themselves head of the household	22	43.9	41.3
Those of these patients, whose husband are head of the household	19	55.6	40.7
Patients who according to Table 3 belong to group C, i.e. up to the end of 1960 acquired at least 2000 sickness points	37	53.8	42.3
Those of these patients who are themselves head of the household	19	48.4	38.0
Those of these patients, whose husbands are head of the household	18	59.6	46.8

Table 54. *Mean ranking figures with regard to standard and size of apartment for different groups of patients included in the material*

Table 55 shows the housing standard in the asthma series each patient being compared with the median person in the total population according to the so called "ranking method" described on page 31 The table distinguishes between heads of the household belonging to the asthma series i e men and unmarried or divorced women and widows, and heads of the household who belong to the rest of the population, i e. men who are married and live with their wives who belong to the asthma series As seen in the table, the asthma series as a whole compared well with the total Uppsala population The median control person in the total population is placed at figure 50 None of the groups shown in the table differed significantly from the expected figure of 50

As seen in Table 56 the mean ranking figure for the housing standard for the total asthma series was 53 6 Those living in the rural districts had a somewhat higher figure and those in the urban districts somewhat lower When the series was divided into two groups, one in which the patients themselves were heads of the household and one in which the patient's husband was the head of the household, a mean figure of 50 was obtained for the former and 56.5 for the latter

For the invalid pensioners included in the series the mean figure was 52 4 and when these were divided into two groups according to whether or not the head of the household was part of the asthma series the figures obtained were 51.2 and 54 0 respectively The corres

Groups acc. to age yrs		Groups according to so-called ranking figures					Mean ranking figure	
		0—20	21—40	41—60	61—80	81—100		
20—34	I	2		2	2		43 0	44 6
	II	1		3			46.	
35—39	I	1		2			52 0	4 4
	II			4			5 4	
40—44	I			1			59 1	61 1
	II			4	1		61 0	
45—49	I	3		3	2		40 3	46.0
	II			2	1		6 8	
50—54	I			1	4	1	66.3	58.3
	II	1	1		9		53 0	
55—64	I	2	6	2	13	1	5 8	5 0
	II	9	1	3	11	2		
65—	I	3	1		4		46 0	4 6
	II	9	5		10		51.	

Table 55 Standard of housing conditions 1960 according to age for each of the individuals in the asthma series expressed in accordance with the so-called ranking method (page 31) where each individual in the asthma series is compared with the so-called median person within the same age and civil status group in the total population (urban and rural district population)

I = head of household included in the asthma series.
II = head of household living with wife who is included in the asthma series.

with water drains w c. and central heating. Of these nine, five were heads of the household belonging to the asthma series eight of the nine were 55 years of age or older

Housing si e

Table 58 show the size of the apartments, according to the number of rooms, of the patients in the whole main asthma series. The tal le is compiled by the ranking method and distinguishes between heads of the household belonging to the asthma series and those belonging to the rest of the population, i. e. men who were married to and lived with women belonging to the asthma series. The table does not give the number of occupants per apartment and thus gives no definite information with regard to overcrowding More accurate information on the occurrence of overcrowding was obtained, however by another method, which will be described below The tal le shows a relatively even distribution of the individuals according to the ranking scale with the reservation, however that in the two younger age groups and in the group of the oldest persons there is some predominance of those whose apartment size was less than that of the median control person.

Groups acc. to age yrs		Groups acc. to standard of housing						
		1 (highest)	2	3	4	5	6	7 (lowest)
30 34	I	1					1	
	II	3	1					
35 39	I	1						
	II	4						
40 44	I	1						
	II	4						
45 49	I	2						
	II	2						
50 54	I	3						
	II	5	1					
55 64	I			1			2	
	II	10		1	1	1		
65-	I	2		1	2			
	II	10		2	1		1	
Total	I	13		2	2		3	
	II	42	2	3	2	1	1	

Table 57 Standard of housing 1960 according to classification (... 51) where the highest standard corresponds to group 1 and the lowest to group 7 for the married Uppsala urban district patient living with wife or husband respectively

I = head of household, included in the asthma series.
II = head of household, living with wife who is included in the asthma series.

ing to the calculation of sickness points, the mean "ranking figure" was 54 8 but here there appeared to be no noteworthy difference between the two groups described above.

Those patients belonging to groups A B and C in Table 3 were studied in Table 56 where it was found that the mean figure for each of the groups exceeded 50 0 It is worthy of note that those heads of the household, belonging to the asthma series, in the groups B and C, i e. the two groups of patients with a relatively high number of sickness points had a mean ranking figure of a little below 50 while those belonging to group A had a remarkably high figure i e 60 None of the groups shown in Table 56 differed significantly from the expected figure of 50 and no significant difference was shown between the pairs of subgroups

The relationship between certain case history data and the housing standard at the time of the investigation was studied Out of the 116 patients the 20 who had shown the highest ranking figures with regard to housing standard and the 20 who had shown the lowest were chosen These two groups of patients were then studied for the purpose of finding out whether any of the eight case history factors mentioned on page 41 (sickness points, duration of disease etc.) had any statistically significant relationship with the housing standard of the patients

The 20 patients who were recorded as having the highest housing standard had a mean ranking figure of 75 6 (range 68 0—84 3) while the 20 with the lowest standard had a mean figure of 13 4 (range 4 0—21 8)

No statistically significant difference was shown between the two groups of patients for any of the factors studied. This meant therefore that with regard to the assessed degree of severity of the disease the 20 patients who were found to have the relatively lowest housing standard did not differ significantly as a group from the 20 with the relatively highest standard.

Table 57 shows the housing standard of the married Uppsala urban district patients, living with wife or husband The classification into groups with different housing standards is based on the conditions shown in Table 54 The age of the women is given as that of their husbands Table 57 allows comparison, within a group homogeneous from the point of view that it consists entirely of married urban dwellers, between the housing standard in the households where the head of the household belonged to the asthma series (the men) and that in the households where the head of the household belonged to the rest of the population (their wives belonging to the asthma series) The table shows that the asthma patients in all the age categories were well placed as a group, with a large majority in the highest standard group None of them were placed in group 7 Of the heads of the household who belonged to the asthma series, 68 1 % lived in apartments belonging to standard group 1 The corresponding figure for the heads of the household living with their wives who belonged to the asthma series was 82.3 %. All except nine had an apartment

units li ing in one room unit. For the married persons in the asthma series, living with their wives or husbands, an expected number of 7.0 with overcrow ded conditions was obtained, while the observed number was 3. This differ ence however was not significant. On calculating the expected value consid eration was taken of the district in which each patient lived, also of civil state and age.

Of the three bserved patient ith over crowded conditions, tw belonged to the urba popul tion nd the third lived in a rural district. Tw of the three patients, one from the tow and one from the country were heads of the household and belonged to the asthma series, while one wa head f the household but belonged to the rest of the population a he wa married to and living with woman who was part of the asthma series. The expected number with overcrowded conditions mong the asthma patients who ere themselves head of the household was 2.8 and the observed number 2.0. The corresponding figures for those patient whose husbands acted head of the household were 4.2 and 1.0 especti ly. When the unmarried persons w lso included in the calculations the umber of expected persons with over crowded onditions increased, while the observed number still remained 1.3.0 since none of those belonging to this category in the asthma series could be regarded as living in overcrowded conditions defined above.

The question of overcrowding among the asthma patients wa also studied o the basis of the definition made by Roos (1949) of the ideal minimal housing standard" i.e. that w th maximum of 1.5 person unit li ing in one room unit. It was then found that 20.7 % of the patients in the sthma series lived under over crowded conditions. For comparison it may be mentioned that Smårs and Ber fenstam (1961) found that the corresponding figure for patients with osteogenesis imperfecta was 26.0 %.

To summari e it may be said that for this group of patients both the standard and the size of the housing were good. When the series of patients was divided into groups according to the assessed degree of severity of the disease no notable difference was shown between these groups. Even the standard of those receiving invalid pensions was equivalent to that of the total population. If anything over crowding occurred to a lesser extent among the patients in the a thma series than in the total population. There was some tendency for those patients whose husbands were head of the household to ha e on an average a somewhat higher standard than those who them selves held this position. When a number of factors found typically in the case histories of asthma patients — were studied, non of them were found to have any statistically significant importance with regard to the housing standard at th tim of the in estigation.

Smoking and alcohol consumption

T bacco smoking The smoking habits of the 116 patients (42 men and 74 women) in the asthma series were studied. Control information was obtained from the *City of Uppsala Health Survey* of 1961 in which 369 men and 4.3 *women were studied.* The method was essentially the same in the two *investigations.* The expected number of *daily smokers* (cigarettes, cigars or

Also shown in the table is the mean number of room units in the different groups according to so called ranking figures The mean number of room units for the whole main series was 2 6

The mean "ranking figure for the housing size in the whole series (see Table 56) was 47.3 for that where the heads of the household belonged to the asthma series 46.3 and for that where the heads of the household belonged to the rest of the population 48 3

For the invalid pensioners included in the series the mean figure was 37 0 the corresponding figure for the non invalid pensioners was 49 0 The difference was not statistically significant Those patients who belonged to groups B and C combined (see Table 56) attained a mean figure of 47.5, while the corresponding figure for those belonging to group A was considerably higher i.e. 57 3 The difference between group A and the combined groups B and C is significant (*) A significant difference was also found between group A and the separate groups B and C.

A study was made of so-called over crowding this being defined in accordance with the population census of 1960 i e as more than 2 0 person

Groups acc to age yrs.		Groups according to so-called ranking figures					Mean ranking figure	
		0—20	21—40	41—60	61—80	81—100		
30—34	I	2	2	2			30	35 3
	II		8		1		42.3	
35—39	I	2	1	2			31 4	36 3
	II		3		1		38.8	
40—44	I					1	91.5	60 2
	II					1	53.4	
45—49	I	1	1	2	1	2	58.8	35 0
	II	1	1			1	46.3	
50—54	I		2	2	1	1	51 0	4 3
	II	1	6		2	2	44.8	
55—64	I	3	0	3	4	4	49.5	51 0
	II	3	5	1	5	3	4 9	
65—	I	3	1	3	1		33.4	41 5
	II	3		8	2	1	44 9	
Mean numbe room units, Tot I + II		1.2	1.8	2.0	3 6	5.2		2 0

Table 58. S e of l ving quarters (No. of room units) 1960 according to age fo each of the ind viduals n th a thma series xpr s ed in accordance with th o-called ranking m thod (page 31) where ach individual in the asthma series i compared with the o-called median person within th same age sex and civil tat group in the t tal population (u ba and rural di tr t popula i n).

I = head of househoud, included in the asthma series.
II = head f household, l ing with wife who is incl ded in the asthma series.

units li ing in one room unit. For the married persons in the asthma series, living with their wives or husbands an expected number of 7.0 with overcrow ded conditions was obtained, while the observed number was 3. This differ ence however was not significant. On calculating the expected value consid eration was taken of the district in which each patient lived, also of ci il state and age.

Of the three observed patient w th over crowded conditions, tw belonged to the urba population nd the third lived in rural district. T of the three patients, one from the tow and one from the country were heads of the household and belonged to the asthma series, while one wa head of the household but belonged to the rest of the population e. he wa married to nd li ing ith ons who w s part of the asthma series. The expected number with overcrowded conditions among the sthma patients who were themselves head of the household wa 3.6, and the observed number 2.0. The corre sponding f gures for those patient whose husbands acted head of the household were 4.2 and 1.0 espectively. When the unmarried persons w re lso included i the calculations the number of expected persons with over crowded ondition increased, while the observed number still remained 3.0, since none of those belonging to this category in the asthma series could be regarded as living in overcrowded cond tions defined abov

Th question of overcrowding among the thma patients was also studied on the basi of the definition mad by Roos (1940) of the ideal minimal housing standard' i.e. that with a maximum of 1.5 person unit living in one room unit. It was then found th t 20.7 % of th patients in the asthma series lived under over crowded conditions. For comparison it may be mentioned that Smårs and Herfenstam (1961) found that the corresponding figure for patients with osteogenesis imperfecta was 20.9 %.

To summari e it may be said that for this group of patients both the standard and the size of the housing were good. When the series of patients was divided into groups according to the assessed degree of severity of the disease no notable difference was shown between these groups. Even the standard of those recei ing invalid pensions was equivalent to that of the total population. If anything over crowding occurred to a lesser extent among the patients in the asthma series than in the total population. There was some tendency for those patients whose husbands were head of the household to have on an average a somewhat higher standard than those who them selves held this position. When a number of factors — found typically in the case histories of asthma patients — were studied none of them were found to have any statistically signifi cant importance with regard to the housing standard at the time of the investigation.

Smoking and alcohol consumption

Tobacco smoking The smoking habits of the 116 patients *(42 men and* 74 women) in the asthma series were studied. Control information was obtained from the City of Uppsala Health Survey of 1961 in which 360 men and 353 women were studied. The method was essentially the same in the two investigations. The expected number of daily smokers (cigarettes, cigars or

pipe) of daily cigarette smokers among this group and also of non smokers were calculated. On calculating these expected figures consideration was taken of sex and age (age group range 5 years)

As seen in Table 59 the number of non smokers was highly significantly greater among the men in the asthma series than among the men in the control material No noteworthy difference was seen in this respect with regard to the women.

Of the men and the women in the asthma series who were daily cigarette smokers, there were none who smoked more than 14 cigarettes per day Of the daily cigarette smokers in the control material a few more than 20 % of the men and a few less than 5 % of the women smoked more than 14 cigarettes per day

Alcohol consumption The alcohol consumption in the 116 patients in the asthma series was studied. As in the study of tobacco smoking the control material consisted of the persons who took part in the City of Uppsala Health Survey In both the asthma series and the control material the groups of "total abstainers and almost total abstainers were selected By almost total abstainers" is meant persons who consume alcoholic drinks including beer less frequently than once a month. The observed number of total and almost total abstainers among the men was 18 while the expected number was 16 The corresponding figures for the women were 57 and 57 respectively In calculating these figures consideration was taken of sex and age groups (group range 5 years)

To summari e the number of tobacco smokers among the men in the asthma series was highly significantly lower than among the men in the control material from the urban district of Uppsala. No noteworthy significance was found in this respect with regard to the women None of the cigarette smokers smoked more than 14 cigarettes per day

In the study of the alcohol con umption no significant difference was shown for the number of total abstainers and almost total abstainers between the asthma series and the control material

	MALE		FEMALE	
	Observed N	Predicted N	Observed No	Predicted No
Non smokers	33	14	30	38
Daily smokers	10	28	15	10
of whom daily cigarette smokers	6	17	15	1

Table 59 Observed and predicted number of tobacco-smokers in the asthma series distributed according to sex.

The significance of difference from the predicted value is stated with significance asterisks.

Discussion

The different parts of the investigation showed that there was surprisingly good social adaptation in this group of patients in spite of the often prolonged and severe disease. Certain factors however should not be forgotten. Perhaps the most important is the large average number of days of incapacity and symptom days reported by these patients. It is obviously impossible to state exactly what this meant in terms of economic burden and reduced production. Some information could certainly be obtained in most cases from the General Sickness Insurance or other sickness benefit societies. But the intermittent nature of the disease points to numerous short periods of illness, resulting in scattered absences from work. This must have affected income and production, and even placed a burden on the finances of the community. Calculations are unnecessary here and furthermore could not provide an accurate answer to these questions. On attempting to assess the economic significance of the disease consideration must be taken of all the journeys made for treatment, the often considerable cost of medicines, and doctors and hospital fees, which affect primarily the patient and secondarily the community. Especially badly affected are obviously those patients supported by invalid pensions. With respect to these persons in particular the burden on the community is also considerable.

It was not considered justified to make a separate description of the effects of the bronchial asthma on the respiratory and circulatory functions in the group of invalid pensioners. The majority of them belonged to the group of patients who were assessed as having the highest degree of severity of the disease, i.e. they had attained 2000 sickness points or more (group C). As expected these patients also had on an average a low ventilation capacity, pronounced signs of hyperinflation and a low physical work capacity while the hemodynamic findings did not differ to any noteworthy degree from the rest of the material or from ordinary conditions. As regards the estimated degree of severity of the bronchial asthma, these patients did not on an average differ notably from the rest of the material.

This series of patients with bronchial asthma was studied from several different aspects. A series of this kind is unavoidably heterogeneous in certain respects. For example, as mentioned previously there were large variations both in the ages of the patients and the ages at which the asthma was first manifested. There were also considerable variations with regard to other factors, for example the duration of the disease up to the time of the investigation. Because of this it was not always possible to use the material in its entirety for studies of the effect of the disease on the social situation of these patients. In the case of family formation, for example, a serious disease occurring at a certain age period may have a determining influence. For several of the factors indicating social adaptation, therefore, studies were made on a part or parts of the material that were considered especially suit

pipe) of daily cigarette smokers among this group and also of non smokers were calculated. On calculating these expected figures consideration was taken of sex and age (age group range 5 years)

As seen in Table 59 the number of non smokers was highly significantly greater among the men in the asthma series than among the men in the control material. No noteworthy difference was seen in this respect with regard to the women

Of the men and the women in the asthma series who were daily cigarette smokers, there were none who smoked more than 14 cigarettes per day Of the daily cigarette smokers in the control material a few more than 20 % of the men and a few less than 5 % of the women smoked more than 14 cigarettes per day

Alcohol consumption The alcohol consumption in the 116 patients in the asthma series was studied. As in the study of tobacco smoking the control material consisted of the persons who took part in the City of Uppsala Health Survey In both the asthma series and the control material the groups of "total abstainers" and "almost total abstainers" were selected. By almost total abstainers is meant persons who consume alcoholic drinks, including beer less *frequently* than once a month The observed number of total and almost total abstainers among the men was 18, while the expected number was 16 The corresponding figures *for the women were 57 and 57 respec*tively In calculating these figures consideration was taken of sex and age groups (group range 5 years)

To summarize the number of tobacco smokers among the men in the asthma series was highly significantly lower than among the men in the control material from the urban district of Uppsala. No noteworthy significance was found in this respect with regard to the women. None of the cigarette smokers smoked more than 14 cigarettes per day

In the study of the alcohol consumption *no significant difference* was shown for the number of "total abstainers" and "almost total abstainers" between the asthma series and the control material

	MALE		FEMALE	
	Observed No	Predicted No	Observed N	Predicted No
Non smokers	32	14	50	38
Daily smokers	10 **	28	15	10
of whom daily cigarette-smokers	6	17	15	17

Table 59 *Observed and predicted number of tobacco-smokers in the asthma series distributed according to sex.*

The significance of difference from the predicted value is stated with significance asterisks.

Discussion

The different parts of the investigation showed that there was surprisingly good social adaptation in this group of patients in spite of the often prolonged and severe disease. Certain factors, however, should not be forgotten. Perhaps the most important is the large average number of days of incapacity and symptom days reported by these patients. It is obviously impossible to state exactly what this meant in terms of economic burden and reduced production. Some information could certainly be obtained in most cases from the General Sickness Insurance or other sickness benefit societies. But the intermittent nature of the disease points to numerous short periods of illness, resulting in scattered absences from work. This must have affected income and production, and even placed a burden on the finances of the community. Calculations are unnecessary here and furthermore could not provide an accurate answer to these questions. On attempting to assess the economic significance of the disease consideration must be taken of all the *journeys* made for treatment, the often considerable cost of medicines, and doctors and hospital fees, which affect primarily the patient and secondarily the community. Especially badly affected are obviously those patients supported by invalid pensions. With respect to these persons in particular the burden on the community is also considerable.

It was not considered justified to make a separate description of the effects of the bronchial asthma on the respiratory functions in the group of invalid pensioners. The majority of them belonged to the group of patients who were assessed as having the highest degree of severity of the disease, i. e. they had attained 2000 sickness points or more (group C). As expected, these patients also had on an average a low ventilation capacity, pronounced signs of hyperinflation and a low physical work capacity, while the hemodynamic findings did not differ to any noteworthy degree from the rest of the material or from ordinary conditions. As regards the estimated degree of severity of the bronchial asthma, these patients did not on an average differ notably from the rest of the material.

This series of patients with bronchial asthma was studied from several different aspects. A series of this kind is unavoidably heterogeneous in certain respects. For example, as mentioned previously there were large variations both in the ages of the patient and the ages at which the asthma was first manifested. There were also considerable variations with regard to other factors, for example the duration of the disease up to the time of the investigation. Because of this it was not always possible to use the material in its entirety for studies of the effect of the disease on the social situation of these patients. In the case of family formation, for example, a serious disease occurring at a certain age period may have a determining influence. For several of the factors indicating social adaptation, therefore, studies were made on a part or parts of the material that were considered especially suit

able. Throughout the whole investigation, however the main interest was concentrated on the question of the *extent to which bronchial asthma had* influenced the possibilities of these persons to adapt themselves socially

A small number of the patients had had relatively few symptom days and days of incapacity *and from a social* adaptation point of view these patients were obviously not as interesting as those who for several years had had frequent more or less prolonged periods of disablement. They were not excluded from the material however *since it was considered best not to* make an exception from the rule that hospital admission during the given period permitted participation in the investigation. Even if the course of their disease was not severe, they had previously had asthma symptoms of *such a degree that admission to hospi*tal was considered necessary It seems probable that the experience of asthmatic attacks and the knowledge that they suffered from a usually prolonged disease might have had some inhibitory influence on the de*velopment of these persons with a* result that their social situation could be affected. *It seemed of interest to* find out whether there were any patients whose condition had improved spontaneously or whether the asthma had possibly changed in character so that the need for urgent hospital care was reduced or no longer existed.

Ask Upmark *(1957)* states that the annual number of deaths from bronchial asthma *in Great Britain* is estimated at 7 per 100 000 inhabitants. The corresponding figure *for the U S.A is 1 6*

Ask Upmark considers it probable that *the mortality* in Sweden is not as high as that in the U S.A., but that the disease gives rise to a great degree of human suffering and that by its recurrent nature and long periods of symptoms it constitutes a handicap to production and national economy Some *information on the mortality* in asthma and related disorders has also been given by Williams (1953) Groen and Lieber (1960) Bower (1961) Mork (1962) and Walkup and Connolly (1963) A number of authors have published series of fatal cases Huber *and Koessler (1922) were among the* first of them. Since this time several workers have discussed deaths from asthma and reviewed the literature, e. g Unger (1952) Earle (1953) Alexander (1958) Williams and Leopold (1959) and Cardell and Pearson *(1959)*

The results obtained from earlier published studies on the *mortality in* asthma contribute only to a small extent to the elucidation of the problems of the present investigation No investigation appears to have been made on the mortality in bronchial asthma alone, where the observations were *made during* a certain period of time and where the observed mortality was compared *with the expected fig*ure, taking into consideration age and sex 15 of the 176 patients *of the pre*sent asthma series died, compared with an expected *figure of 10.3 If the* five cases in whom an actual asthmatic attack was considered to be the direct cause of death are excluded there remain ten deaths, which is almost equal to the expected number This in

dicates — if the cases in whom the asthma in itself was considered to be the direct cause of death are disregarded — that the disease did not have had the indirect effect of increasing the mortality.

No definite reason for the relatively small tendency of the asthma patients to move *to other districts* can be given, but there were probably several contributory factors. It is reasonable to assume that patients with this disease avoid taking certain risks. Moving from one place to another is often connected with a change of employment. For a person with a chronic disease there is probably often little attraction in the idea of taking the risks that may be associated with moving, when this means leaving a milieu and place of employment that he has perhaps been accustomed to for a long period.

For many patients moving would also mean losing contact with the doctor upon whom he might have relied for several years. The knowledge that his own doctor and possibly the hospital that has essential data, would probably be available in an emergency is certainly of great importance to many asthma patients.

On the other hand it would not have been surprising to find that these patients had moved to a greater extent than the rest of the population. The change of climate which since of old has often been recommended to persons suffering from asthma is a factor to be considered, but this does not appear to have been of any importance in this series of patients. The reason may possibly be — which was often evident from discussions with the patients — that for many patients occasional visits to other places did not prove to have the desired effect on their asthma.

Another reason for expecting that these patients might have a greater tendency to move to other places is that this disease might be expected to predispose the patient to a change of occupation. This factor appeared to be of little importance in this series, however possibly due to the fact, as mentioned previously, that moving to another district often entails certain risks, and also to the fact that the labour market was favourable and other work was therefore available at closer quarters.

It is natural for older persons to feel so settled in their milieu that they do not often entertain the idea of moving. The results of the present study showed, however, that it was not only among the older patients that there was a high frequency of those who continued to live in Uppsala. Thus it was not predominance of the older age groups that gave the relatively high frequency of those who continued to live in Uppsala, in the asthma series as a whole.

Part of the material was studied with regard to *general education*. For this study only those patients who had symptoms of asthma at the ages of 16—24 years (*see page 122*) were included. Here, as in the study of the effect of the asthma on the formation *of families, the material* was small, but it was nevertheless decided to study this factor in order to shed as much

light as possible on the significance of the disease from the social adaptation point of view

No significant difference was found with regard to the number of lower certificate and matriculation examinations passed between the asthma series and the total population There was a possibly tendency to a somewhat greater number of examinations passed among the asthma patients. There appears therefore to be some support for the assumption that these patients who had symptoms of asthma at the ages 16—24 years regarded as a group had just as good a general education as the total population The reason that the patients had, on an average such a good education could of course have been that they had special intellectual or social prerequisites There is, however no support for such an assumption. The reason may also possibly be that these patients or their parents, in their effort to avoid the type of work in which there was greater demand on physical strain chose training of a more intellectual nature. On the other hand there were probably some pupils who because of the severity of the disease had difficulty in attending the school lessons to a sufficient extent, and were therefore more badly situated in the competition for marks and examinations. In the individual asthma patients it was seen that their development was influenced sometimes more by one and sometimes more by another of these factors, and for the group as a whole the factors causing difficulties appeared to be more than well compensated.

It was mentioned on giving the results of this study that the favourable conditions for education in Uppsala may have influenced the number of lower certificate and matriculation examinations passed by patients in this series. The significance of this good access to education was reduced somewhat for this material however by the fact that some of the patients studied did not move to Uppsala until after the age of 18 years In the investigation made on the number of patients who had a university education or its equivalent however the control material consisted of the total population of Uppsala, and a direct comparison between the asthma series and the control material was not therefore affected by the advantages of the local educational conditions

The number of patients in the asthma series who were or had been married agreed well with the expected figure which was calculated with regard to sex and age and also to district i e. whether the patient belonged to the urban or rural population — this because the marriage frequency particularly for the men, was lower in the rural districts than in the town. Since in a large number of the patients in the asthma series the onset of the disease occurred after the age of 30 years — the age when the majority of all marriages have already taken place — it was considered more suitable in studying the effect of the asthma on the marriage frequency to select a material of patients in whom the asthma was first manifested at or before the age of 21 years and who during the ages 20—29 years had no more than two symptom free years In this group

also there was very good agreement between the observed and the ex pected numbers of those who had at any time been married. It had appear ed possible here that for these patients the asthma might have constituted a burden both with regard to meeting members of the opposite sex and in the question of liabilities for maintenance. The fact that these assumptions were not supported by the results may be connected with the usually intermittent course of the disease which may in clude periods when the symptoms are absent or only mild.

This factor might also be expected to be of importance with regard to the attitude of the patients towards the greater maintenance burden that accompanies the addition of children to the family The group tudied here consisted of married patients, living with their husbands or wives — in age groups where it may be assumed that the overwhelming majority of the children are below the age of 16 years. In these selected patients the asthma was first manifested at or before the age of 21 years, and during the ages 20—29 years they had had at the most two symptom-free years No difference wa shown with regard to the *number of childr n* under the age of 16 years in a comparison with th correspond ing age groups in the total populations of the urban and rural districts resp ecti ely It would have been under tandable if this figure had been lower in th asthma group when considering that for the men the responsibility of upporting a family might have con tituted an nhibitory factor to having children, and that for the women it is conceivable that fear of manifestations of the disease might have caused some fear of pregnancy and delivery

The tendency to fewer *divorced* persons in the asthma series than in the total population was relatively marked the difference between the observed and expected numbers of those who had at any time been di vorced was statistically significant. Bruce Dahlström and Uggla (1960) showed in a study of tuberculous pati ents that a remarkably large number were single or divorced. Of the men of ages 15—65 years no fewer than 25 % belonged to the former and just as many to the latter category The cor responding figures for the total popul ation were given as 10 % single pers ons and 7 % divorced.

There are however some striking differences between the two series which must first be emphasized The frequently large consumption of alco hol among the tuberculous patients, the long periods of hospital care often needed by patients with the disease, and the biased ideas till associated with this diagnosis are some examples indicating that direct comparison with asthma patients is not justifiable in all respects.

It might thus seem surprising that the number of patient in the asthma series living as divorced was fewer than the expected number It is only possible to present a few suppositions as an explanation. Bronchial asthma is a prolonged and intermittent disease, that usually requires lots of consulta tions and repeated short periods of hospital care, and it is possible that in reality this may lead to a high degree

light as possible on the significance of the disease from the social adaptation point of view

No significant difference was found with regard to the number of lower *certificate and matriculation* examinations passed between the asthma series and the total population There was a possibly tendency to a somewhat greater number of examinations passed among the asthma patients. There appears therefore to be some support for the assumption that these patients who had symptoms of asthma at the *ages 16—24 years, regarded as a group* had just as good a general education *as the total population The reason* that the patients had, on an average, such a good education could of course have been that they had special intellectual or social prerequisites There is, however no support for such an assumption. The reason may also possibly be that these patients or their parents in their effort to avoid the type of work in which there was greater demand on physical strain chose training of a more intellectual nature. On the other hand there were *probably some pupils who because of* the severity of the disease had difficulty *in attending the school lessons* to a sufficient extent, and were therefore more *badly situated in the competition* for marks and examinations In the individual asthma patients it was seen that their development was influenced sometimes more by one and sometimes more by another of these factors and for the group as a whole the factors causing difficulties appeared to be more than well compensated

It was mentioned on giving the results of this study that the favourable *conditions for education in Uppsala* may have influenced the number *of lower certificate* and matriculation examinations passed by patients in this series The significance of this good access to education was reduced somewhat for this material, however by the fact that some of the patients studied did not move to Uppsala until after the age of 18 years. In the investigation made on the number of patients who had a *university* education or its equivalent however the con*trol material consisted of the total* population of Uppsala, and a direct *comparison between the asthma series* and the control material was not therefore *affected by* the advantages of the local educational conditions

The number of patients in the asthma series who were or had been married agreed well with the expected figure which was calculated with regard to sex and age and also to district i e whether the patient belonged to the urban or rural population — this because the *marriage frequency* particularly for the men was lower in the *rural districts than* in the town. Since in a large number of the patients in *the asthma series* the onset of the disease occurred after the age of 30 years — *the age when the majority* of all marriages have already taken place — *it was considered more* suitable in studying the effect of the asthma on the marriage frequency to select a material of patients in whom the asthma was *first manifested* at or *before* the age of 21 years and who during the ages 20—29 years *had no more than* two symptom free years In this group

also there was very good agreement between the observed and the expected numbers of those who had at any time been married. It had appeared possible here that for these patients the asthma might have constituted a burden both with regard to meeting members of the opposite sex and in the question of liabilities for maintenance. The fact that these assumptions were not supported by the results may be connected with the usually intermittent course of the disease which may include periods when the symptoms are absent or only mild.

This factor might also be expected to be of importance with regard to the attitude of the patients towards the greater maintenance burden that accompanies the addition of children to the family The group studied here consisted of married patients, living with their husbands or wives — in age groups where it may be assumed that the overwhelming majority of the children are below the age of 16 years. In these selected patients the asthma was first manifested at or before the age of 21 years, and during the ages 20—29 years they had had at the most two symptom free years. No difference was shown with regard to the number *of children* under the age of 16 years in a comparison with the corresponding age groups in the total populations of the urban and rural districts respectively It would have been understandable if this figure had been lower in the asthma group when considering that for the men the responsibility of supporting a family might have constituted an inhibitory factor to having children, and that for the women it is conceivable that fear of manifestations of the disease might have caused some fear of pregnancy and delivery

The tendency to fewer *divorced* persons in the asthma series than in the total population was relatively marked the difference between the observed and expected numbers of those who had at any time been divorced was statistically significant. Bruce Dahlström and Uggla (1960) showed in a study of tuberculous patients that a remarkably large number were single or divorced. Of the men of ages 45—65 years no fewer than 25 % belonged to the former and just as many to the latter category The corresponding figures for the total population were given as 10 % single persons and 7 % divorced.

There are, however some striking differences between the two series which must first be emphasized The frequently large consumption of alcohol among the tuberculous patients, the long periods of hospital care often needed by patients with the disease and the biased ideas still associated with this diagnosis are some examples indicating that direct comparison with asthma patients is not justifiable in all respects.

It might thus seem surprising that the number of patients in the asthma series living as divorced was fewer than the expected number It is only possible to present a few suppositions as an explanation. Bronchial asthma is a prolonged and intermittent disease that usually requires lots of consultations and repeated short periods of hospital care, and it is possible that in reality this may lead to a high degree

light as possible on the significance of the disease from the social adaptation *point of view*

No significant difference was found with regard to the number of lower certificate and matriculation examinations passed between the asthma series and the total population. There was a possibly tendency to a somewhat greater number of examinations passed among the asthma patients There appears therefore to be some support for the assumption that these patients who had symptoms of asthma at the ages 16—24 years regarded as a group had just as good a general education as the total population. The reason that the patients had on an average such a good education could of course have been that they had special intellectual or social prerequisites There is, however no support for such an assumption The reason may also possibly be that these patients or their parents, in their effort to avoid the type of work in which there was greater demand on physical strain chose training of a more intellectual nature. On the other hand there were probably some pupils who because of the severity of the disease had difficulty in attending the school lessons to a sufficient extent, and were therefore more badly situated in the competition for marks and examinations. In the individual asthma patients it was seen that their development was influenced sometimes more by one and sometimes more by another of these factors, and for the group as a whole the factors causing difficulties appeared to be more than well compensated.

It was mentioned on giving the results of this study that the favourable conditions for education in Uppsala may have influenced the number of lower certificate and matriculation examinations passed by patients in this series. The significance of this good access to education was reduced somewhat for this material, however by the fact that some of the patients studied did not move to Uppsala until after the age of 18 years. In the investigation made on the number of patients who had a university education or its equivalent, however the control material consisted of the total population of Uppsala and a direct comparison between the asthma series and the control material was not therefore affected by the advantages of the local educational conditions.

The number of patients in the asthma series who were or had been married agreed well with the expected figure, which was calculated with regard to sex and age and also to district, i e whether the patient belonged to the urban or rural population — this because the *marriage frequency* particularly for the men, was lower in the rural districts than in the town. Since in a large number of the patients in the asthma series the onset of the disease occurred after the age of 30 years — the age when the majority of all marriages have already taken place — it was considered more suitable in studying the effect of the asthma on the marriage frequency to select a material of patients in whom the asthma was first manifested at or before the age of 21 years and who during the ages 20—29 years had no more than two symptom free years. In this group

to stop working or his employer asked him to leave at least for a time. When later the asthma reached a calmer stage there was not always need for the patient at his old place of employment and he had to obtain a new job, but there was often an opportunity here of choosing work of a type more suitable with regard to the disease. This situation probably applied to a greater extent to the less qualified persons than to the more highly qualified. Other patients changed their occupations for preventive reasons — often to avoid certain factors that had previously acted provokingly on the latent asthma.

The frequency of *economically active married women* was on the whole the same in the asthma series as in the corresponding population. It must be regarded as remarkable that this group of women including many who for several years had been completely or partly disabled by asthma, were now to be found to such a great extent among those who were carrying out income yielding work. In many of them the disease had improved during the latter years and this had provided an opportunity of breaking the isolation that had previously been unavoidable on account of repeated frequent periods of asthma symptoms. This probably explains, at least partly, the fact that such a relatively large number of these women were found to be working. It was gratifying to find that this form of readaptation was possible for several women in spite of the fact that often only one or a *few years* had elapsed since a time when the asthma was so severe that income-yielding work was almost out of the question.

It may be assumed that the distribution into sickness benefit classes by the General Sickness Insurance gives a good idea of the differences in the mean *income levels* of groups of individuals. The information given by the members to the General Sickness Insurance with regard to their income is checked to a great extent with that given to the income tax authorities.

On studying the income level in the group of economically active men and women in 1963, it was found that the mean income level in December of that year was for the men practically the same as that of the men in the control material. The women in the asthma series, on the other hand, had on an average a somewhat lower income level than expected.

It was found that eight years earlier i. in December 1955 the income level of the men in the asthma series, born in 1897 or later who in 1963 were not receiving an invalid pension and were not self-employed, seemed to be lower than that of the corresponding median person in the total population by an average of about 20 %, consideration being taken of age, sex and district of residence. In December 1960 this group of patients had caught up to such an extent that the difference was reduced by about half and after a further three years the income level of the median person in the control material had been reached on an average by these patients in the asthma series. Even if this tendency to an improvement in income did not reach the significance level it appeared to correspond well with the

of solidarity and unity between the husband and wife. It is certainly possible that the situation of the patient with attack *after attack of* severe asthma could produce a *strong feeling* of sympathy in the husband or wife The patient, on his side, is obviously in a situation that is often characterized by *need of help* whether this be assistance during acute *attacks or long term help* e g main tenance etc.

The distribution between the different *branches of industry* among the economically active patients in the *asthma series offered* no indication that these individuals differed from the rest of the population in this respect. The distribution of the economically active patients between the two *occupational positions* of employer and employee showed an over representat ion in the former group for the asthma series when compared with the total population. One possible contributory explanation is that the employer with his more independent position is able to plan his work *with regard to his* disease and that handicapped persons often therefore choose this form of position The fact that the number of persons receiving invalid pensions was slightly *greater among* the group of em ployees in this material also supports the view *that* the employer *may have* greater possibilities of reconciling the disease with his work. A tendency to over representation in the group of employers was also found in the study *by Smårs* and Berfenstam (1961) of patients with osteogenesis imperfecta and it is *highly probable* that this ef fort to obtain work of an independent nature is characteristic of handicapped persons

The social consequences of asthma *are seen* perhaps *especially* clearly *by* the fact that approximately every fourth man in the present series was forced to *discontinue income-yielding work* permanently on account of asth*ma and furthermore that approxim*ately every third man had to *change his occupation* for the same reason. A notable number of the men had left agricultural and *forestry* work, either when they *permanently stopped* work ing or on changing their occupations. *Since the actual distribution of the* economically active men in the asthma series appeared to indicate some degree of over representation in these branch es of industry this means that a large *number of the men* earned or had previously earned their living from agriculture and forestry work. This may seem reasonable — from a causa tive aspect — in consideration of the special position of this type of work with regard to allergies.

Several of the women in the material were also affected by the disease in a similar way *but not however* to the same extent numerically as the men since the women did not have steady permanent work to the same extent as the men Further the social conse quences in the women were probably of less significance than in the men, *since the latter are more often* respon sible for supporting the family

The reason *that asthma necessitated* changes in occupation was in some cases that the disease had reduced the working capacity to such an extent that either the patient *himself* decided

unity of studying the effect of the patient's asthma on her family. It is conceivable that this latter effect may be expressed in the fact that a relatively *large proportion of the economic* resources of the family is allotted to the home. If this was in fact so it would support the view that the asthma influenced these patients or their families to improve their housing standard.

It may also be asked whether special financial benefits from the community which may be received by some patients, e. g. invalid pensioners, could have contributed to the good average housing standard. A further contributory factor may be that the disease demanded a relatively large degree of assiduity. This could perhaps have contributed partly to the fact that the patient had been able to retain an apartment of good class, or to obtain a different one of a better standard than the old. It may however be mentioned that for the invalid pensioners the average *difference from* the housing *size for the median person* in the total population almost reached the limit of significance.

In presenting the results of the majority of the studies on different indications *of social adaptation, those 12* patients who had other chronic diseases — in addition to the *asthma* — which could have h d some determining influence on his lif in general, his health condition and functions were excluded. This group of patients did not differ appreciably from the rest of the material however with regard to the indicators studied.

It is obvious *that to a group of pati*ents such as those in the asthma series the possibilities of good medical care and general support in cases of employment difficulties or disablement, offered by the community must have been of significance with regard to their social situation. The fact that the two decades preceding the time of the *present investigation* were on the whole characterized by a favourable labour market contributed to the relatively good opportunities of the handicapped to assert themselves in the competition for employment. This factor applied especially to the economically active persons, and less to those receiving invalid pensions. It was obvious that in the case of many of the latter persons the support of the community the family relatives and others was of decisive importance.

Whether this series of patients had on an average better intellectual prerequisites than the total population was not clear Certain factors appear to indicate that the disease resulted in some concentration on education and *professional training with an aim at* more favourable positions, and it is possible that this could also be connected with the fact that the disease demanded an assiduous manner of *living* No psychiatric investigations were made on this material but on the many occasions of personal contact and often detailed conversations with the patients mainly during the collection of the material, they gave on the whole the impression of good mental balance

The results obtained from the studies of the different indicators used to give

improvement as regards the asthma noted for the majority of the patients on taking their case histories, consideration being taken of the estimated number of days of incapacity due to asthma and days with asthma symptoms during earlier periods

Increased prosperity in a community is to a great part expressed by a raised *standard of housing* which may probably be said to constitute an important measure of the social situation of a person or group of persons This is not only because in the majority of cases the housing standard of a person is related to his income end economic situation, but also because generally speaking this standard constitutes some indication of his ambition to organize a satisfactory milieu for himself and his family Even when the asthma material was divided into sub-series so that the mean housing standard for certain groups that were especially badly affected by the asthma e. g invalid pensioners, was studied separately it was shown that these groups compared relatively well with the total population, even if there was often some difference between those patients in the series who were themselves heads of the household and those whose husbands held this position The latter category constantly showed a somewhat higher mean housing standard than the corresponding total population which appeared to indicate that those families in which the wife had asthma made a special endeavour to keep up a good standard. It seems to be rather more remarkable that the group of invalid pensioners on an average did not have a lower housing standard than the total population. When, however a study was made of those persons who at the time of the housing census (1960) had the highest mean number of sickness points per year (1957—61) it was found that this group on an average showed the lowest housing standards of the asthma series Not for these patients either however was the average difference from the housing standard reached by the median person in the total population particularly large. When the influence of a number of factors — found typically in the case history of asthma patients — were studied none of them were found to have any statistically significant importance with regard to the housing standard at the time of the investigation.

It may be asked whether the frequently discussed relationship between asthma and the housing standard (damp draughts etc.) may constitute some reason for the good housing situation found on an average among these patients. In other words do asthma patients often make a special effort to obtain good housing conditions in the hope that this will have a favourable effect on the course of the disease? Conversations with the patients in this series indicated that this was true for several of them Some support for this supposition appears to be offered by the fact mentioned above that there was some tendency — not however significant — to a relatively high housing standard in the group of married women of the series, living with their husbands. The study of this group is of especial interest in this respect since to some extent it provides an opport

unity of studying the effect of the pati ent's asthma on her family. It is conceivable that this latter effect may be expressed in the fact that a *relatively* large proportion of the economic resources of the family is allotted to the home. If this was in fact so, it would support the view that the asthma influenced these patients or their families to improve their housing standard.

It may also be asked whether special financial benefits from the community which may be received by some patients, e. g. in alid pensioners, could have contributed to the good average housing standard. A further contributory factor may be that the disease demanded a relatively large degree of assiduity. This could perhaps have contributed partly to the fact that the patient had been able to retain an apartment of good class, or to obtain a different one of a better standard than the old. It may however be mentioned that for the invalid pensioners the average difference from the hou ing size for the median person in the total population almost reached the limit of significance.

In presenting the results of the majority of the studies on different indications f social adaptation, those 12 patient who had other *chronic diseases* — in addition to the asthma — which could have had some determining influence on his life in general, his health condition and functions were excluded. This group of patients did not differ appreciably from the rest of the material, however with regard to the indicators tudied.

It is obvious that to a group of pati ents such as those in the asthma series the possibilities of good medical care and general support in cases of employment difficulties or disablement, of fered by the community mu t have been of significance with regard to their social situation. The fact that the two decades preceding the time of the present investigation were on the whole characterized by a favourable labour market contributed to the relatively good opportunities of the handicapped to assert themselves in the competition for employment. This factor applied especially to the economically active persons, and less to those receiving in valid pensions. It was obvious that in the ca e of many of the latter persons the support of the community the family relatives and others was of decisi e importance.

Whether this series of patients had on an average better intellectual prerequisites than the total population was not clear. Certain factors appear to indicate that the disease resulted in some concentration on education and professional training with an aim at more *favourable* positions, and it is possible that this could also be connected with the fact that the disease demanded an assiduous manner of living. No psychiatric investigations were made on this material, but on the many occasions *of* personal contact and often detailed conversations with *the patients mainly during* the collect ion of *the material, they* gave on the whole the impression of good mental balance.

The results obtained from the studies of the different indicators used to give

improvement as regards the asthma noted for the majority of the patients on taking their case histories, consideration being taken of the estimated number of days of incapacity due to asthma and days with asthma symptoms during earlier periods

Increased prosperity in a community is to a great part expressed by a raised *standard of housing* which may probably be said to constitute an important measure of the social situation of a person or group of persons This is not only because in the majority of cases the housing standard of a person is related to his income end economic situation but also because generally speaking this standard constitutes some indication of his ambition to organize a satisfactory milieu for him self and his family Even when the asthma material was divided into subseries so that the mean housing standard for certain groups that were especially badly affected by the asthma e. g invalid pensioners, was studied separately it was shown that these groups compared relatively well with the total population, even if there was often some difference between those patients in the series who were themselves heads of the household and those whose husbands held this position. The latter category constantly showed a somewhat higher mean housing standard than the corresponding total population which appeared to indicate that those families in which the wife had asthma made a special endeavour to keep up a good standard. It seems to be rather more remarkable that the group of invalid pensioners on an average did not have a lower housing standard than the total population. When however a study was made of those persons who at the time of the housing census (1960) had the highest mean number of sickness points per year (1957—61) it was found that this group on an average showed the lowest housing standards of the asthma series Not for these patients either however was the average difference from the housing standard reached by the median person in the total population particularly large. When the influence of a number of factors — found typically in the case history of asthma patients — were studied, none of them were found to have any statistically significant importance with regard to the housing standard at the time of the investigation.

It may be asked whether the frequently discussed relationship between asthma and the housing standard (damp, draughts etc.) may constitute some reason for the good housing situation found on an average among these patients In other words do asthma patients often make a special effort to obtain good housing conditions in the hope that this will have a favourable effect on the course of the disease? Conversations with the patients in this series indicated that this was true for several of them Some support for this supposition appears to be offered by the fact, mentioned above, that there was some tendency — not, however significant — to a relatively high housing standard in the group of married women of the series, living with their husbands. The study of this group is of especial interest in this respect, since to some extent it provides an opport

ssibility of studying the effect of the patient's asthma on her family. It is conceivable that this latter effect may be expressed in the fact that a relatively large proportion of the economic resources of the family is allotted to the home. If this was in fact so, it would support the view that the asthma influenced these patients or their families to improve their housing standard.

It may also be asked whether special financial benefits from the community which may be received by some patients, e. g. invalid pensioners, could have contributed to the good average housing standard. A further contributory factor may be that the disease demanded a relatively large degree of assiduity. This could perhaps have contributed partly to the fact that the patient had been able to retain an apartment of good class, or to obtain a different one of a better standard than the old. It may however be mentioned that for the invalid pensioners the average difference from the housing size for the median person in the total population almost reached the limit of significance.

In presenting the results of the majority of the studies on different indications of social adaptation, those 12 patients who had other chronic diseases — in addition to the asthma — which could have had some determining influence on his life in general, his health condition and functions were excluded. This group of patients did not differ appreciably from the rest of the material, however, with regard to the indicators studied.

It is obvious that to a group of patients such as those in the asthma series the possibilities of good medical care and general support in cases of employment difficulties or disablement offered by the community must have been of significance with regard to their social situation. The fact that the two decades preceding the time of the present investigation were on the whole characterized by a favourable labour market contributed to the relatively good opportunities of the handicapped to assert themselves in the competition for employment. This factor applied especially to the economically active persons and less to those receiving invalid pensions. It was obvious that in the case of many of the latter persons the support of the community, the family, relatives and others was of decisive importance.

Whether this series of patients had on an average better intellectual prerequisites than the total population was not clear. Certain factors appear to indicate that the disease resulted in some concentration on education and professional training with an aim at more favourable positions, and it is possible that this could also be connected with the fact that the disease demanded an assiduous manner of living. No psychiatric investigations were made on this material but on the many occasions of personal contact and often detailed conversations with the patients mainly during the collection of the material, they gave on the whole the impression of good mental balance.

The results obtained from the studies of the different indicators used to give

an idea of the adaptation to the community of this group of patients, provide a perhaps unexpectedly bright picture. It must be remembered, however on reviewing the effects of this disease, that a certain degree of excess mortality — but not significant — has been shown. It must also be remembered that from a social point of view the effects of this disease were felt to a great extent by many patients. No fewer than every fifth patient had received an invalid pension on account of asthma.

Summary

No *systematic socio-medical* investigations on the effects of bronchial asthma on the adaptation of the patient to the community appear to have been made previously. A report is made here of a socio-medical study of persons who were still living in Uppsala and its surrounding rural districts after a certain period of time and who had been previously admitted to an Uppsala hospital for the treatment of bronchial asthma. In some parts of the investigation, namely the study of the *frequency of changes of residence and* the mortality study those who had moved to other districts were also included. 128 patients were interviewed and examined personally by the author. 12 of them had or had had other chronic bronchial diseases, apart from the bronchial asthma, and these patients were therefore not included in the general analyses of the results of studies of different indicators of social adaptation.

Some degree of *excess mortality* was shown but this was not significant. Of the 15 patients in this series who died (expected number 10.3) it was considered that 5 had died as a direct result of asthmatic attacks. If those cases are disregarded, in which the bronchial asthma *in itself* was considered to be the direct cause of death the disease thus did not appear to have had the indirect effect of increasing the mortality rate. Approximately every fifth patient was receiving an *invalid pension* solely or mainly by reason of asthma. These patients constituted a group in which both the patient himself and the community were considerably burdened by the social consequences of the disease.

For the purpose of finding out the extent to which these patients *moved to other districts* a study was made on those who, on admission to hospital were living in the Uppsala urban district. *The factors that might be* assumed to influence the patients to remain in their own district or move to another ... The standard weigh... still living i... signi... ies th... contr... perso... at r... ule.

T...

in...

ents,

was manifested at or before the age of 16 years, appeared satisfactory when assessed by the number of lower school certificate and matriculation examinations passed. No noteworthy difference was shown between the asthma series and the total population as regards the number of persons with a university education. This comparison was based on information from official statistics, including the population census of 1960. Some factors that might be expected to inhibit or promote education in a group of patients such as this were discussed.

The *marriage frequency* was of the same order of size as that in the total population. This also held for the small number of patients in whom the asthma was manifested at or before the age of 21 years and who suffered from symptoms of this disease during the ages 21—30 years. In a study of the men and women in this latter group, who were born in 1921 or later and 1924 or later respectively — i.e. the age groups in which the overwhelming majority of children were below 16 years of age — it was found that th *numbe f children* below that age did not differ significantly from the expected number. The patients in the asthma series showed a relatively marked tendency to *fewer divorces* than the total population. For the studies of the marriage frequency, number of children and number of divorces, information for the total population was obtained from the 1960 population census. For the number of divorces information was also obtained from the registrar's office.

The distribution between the different *branches of industry* among the economically active patients of the asthma series gave no indication that these patients differed from the total population in this respect. A study of the distribution between the two *occupational positions* of employer and employee showed an over-representation in the group of employers among the patients in the asthma series when compared with the total population. In these two studies also, information was obtained from the 1960 population census. Over half of the men found it necessary either to *discontinue income yielding work or change their occupation* on account of asthma, a fact which in itself strongly emphasizes the way in which this disease affected many patients. A report is made of the changes of occupation among these patients, and the reasons are discussed. There was an obvious tendency for the patients to change over to work involving less physical strain.

The frequency of *married women with income-yielding work* agreed in the main with that in the corresponding total population, in spite of the fact that the working capacity of many of the women in the asthma series had often been greatly reduced for several years.

The *income level* of the economically active patients in the asthma series was studied with the aid of the classification made by the General Sickness Insurance into sickness benefit groups. No noteworthy difference was found between the men and the control material. The women, however appeared to have a lower income level. A study was made of the *income devel*

an idea of the adaptation to the community of this group of patients, provide a perhaps unexpectedly *bright picture*. It must be remembered, however on reviewing the effects of this disease, that a certain degree of excess mortality — but not significant — has been shown. It must also be remembered that from a social point of view the *effects* of this disease were felt to a great extent by many patients. No fewer than every fifth patient had received an invalid pension on account of asthma.

Summary

No systematic socio-medical investigations on the effects of bronchial asthma on the adaptation of the patient *to the community appear* to have been made previously. A report is made here of a socio-medical study of persons who were *still living* in Uppsala and its surrounding rural districts after a certain period of time and who had *been previously admitted to* an Uppsala hospital for the treatment of bronchial asthma. In some parts of the *investigation, namely the study of the* frequency of changes of residence and the mortality study, those who had *moved to other districts* were also included. 128 patients were interviewed and examined personally by the author. 12 of them had or had had other chronic bronchial diseases, apart from the bronchial asthma, and these patients were therefore not included in the general analyses of the results of studies of different indicators of social adaptation.

Some degree of *excess mortality* was shown but this was not significant. Of the 15 patients in this series who died (expected number 10.3) it was considered that 5 had died *as a direct result* of asthmatic attacks. If those cases are disregarded, in which the bronchial asthma in itself was considered to be the direct cause of death, the disease *thus did not appear to have had the* indirect effect of increasing the mortality rate. Approximately every fifth patient was receiving an *invalid pension* solely or mainly by reason of asthma. These patients constituted a group in which both the patient himself and *the community were considerably* burdened by the social consequences of the disease.

For the purpose of finding out the extent to which these patients *moved to other districts* a study was made on those who, on admission to hospital, were living in the Uppsala urban district. The factors that might be assumed to influence the patients to remain in their own district or move to another were discussed. The standard weighted percentage of those still living in Uppsala after a given period was *significantly higher in the asthma series* than in the total population. The control material comprised every 36th person in Uppsala, these being selected at random from the population schedule.

The *general education* which was studied in a small number of the patients viz. *those in whom the asthma*

IX GENERAL SUMMARY

The aim of this investigation was to study the long term effect of bronchial asthma on respiratory and circulatory functions and on social adaptation, in relation to the estimated degree of severity of the disease.

The degree of severity was assessed by the approximate number of days of incapacity days with symptoms number of hours with asthmatic attacks (attack hours) etc

Lung volumes, entilation capacity physical work capacity and hemodynamic functions were studied during periods in which the condition of the patients as regards their asthma was optimal. Comparisons were made both between diff rent groups, after classification according to the estimated degree of severity of the asthma, and between the series of asthma patients studied nd control ubjects.

With regard to ventilation capacity the mean intensity of the asthma during the fi e years almost immediately preceding th test of thi function appeared to ha e greater influence on the result than the o er all degree of severity since the onset of this disease On the other hand the increase in the ratio of the residual volum (RV) to the total lung capacity (TLC) a measure of hyperinflation, appears to depend mainly on the degree of severity of the disease as estimated for the whole period since it was first manifested. It is plausible that the reduction in ventilation capacity — which can be demonstrated in free intervals — is to some extent of a reversible nature. Reversibility appeared to be valid to a lesser extent for the state of hyperinflation. It is supposed that hyperinflation — although an at least partly irreversible sign in the present series — does not necessarily imply a stat of emphysema (defined as a condition complicated by alveolar wall destruction)

A significant correlation was found for the patients in the present series between ventilation capacity and physical work capacity The partial correlation between physical work capacity and the estimated degree of severity of the bronchial asthma — when the influence of the reduction of ventilation capacity has been eliminated — was low This indicates that the influence of the bronchial asthma on the physical work capacity was exerted to the greatest part via the influence on the ventilation capacity The relationship between the ratio RV/TLC and physical work capacity was found to be of no appreciable importance.

opment from 1955 to 1963 in the employed male persons in the asthma series this was also based on the classifications made by the General Sickness Insurance. Compared with the control material the mean income level of the men in the asthma series appeared if anything to have improved to a somewhat greater extent but this difference was not significant.

It was of especial interest to study the *housing standard* of the asthma patients, not only because for the majority of people this is related to their income and economic situation but also because it may be generally said that this standard gives some idea of the ambition of a person to organize a satisfactory milieu for himself and his family i e his efforts at social adaptation This study was also of interest in as much as this disease and its course are often said to be associated with the housing standard. The average housing standard of the asthma patients was good. No noteworthy difference was found in this respect between these patients and the total population. Even when the asthma material was divided into sub-series so that the mean standard could be studied in groups that were especially badly affected by the disease e. g the invalid pensioners, there appeared to be no great difference in housing standard between these groups and the total population The information with regard to the total population was obtained from the 1960 population census.

The *tobacco-smoking and alcohol consumption habits* were studied. There appeared to be good agreement both for the men and the women with regard to the observed and expected numbers of total and almost total abstainers. The expected and observed numbers of non smokers among the women were very similar With regard to the men a highly significantly smaller number of smokers was found in the asthma series, and the amount of tobacco used by these smokers was less than that used by the smokers in the control material The expected values for the patients in the asthma series were calculated from information obtained from the City of Uppsala Health Survey of 1961

The results obtained in the studies of the different indications of adaptation to the community show a perhaps unexpectedly bright picture It must be pointed out however that in order to obtain an adequate over all impression consideration must be taken of the number of patients — relatively small it is true — who had died of their asthma. The large average number of days of incapacity and symptom days reported by the patients should also be taken into account as well as the fact that in the group of invalid pensioners in particular the disease had often had deeply felt consequences

IX. GENERAL SUMMARY

The aim of this investigation was to study the long term effect of bronchial asthma on respiratory and circulatory functions and on social adaptation, in relation to the estimated degree of severity of the disease.

The degree of severity was assessed by the approximate number of days of incapacity days with symptoms, number of hours with asthmatic attacks ("attack hours") etc.

Lung volumes, ventilation capacity physical work capacity and hemodynamic functions were studied during periods in which the condition of the patients as regards their asthma was optimal. Comparisons were made both between different groups, after classification according to the estimated degree of severity of the asthma, and between the series of asthma patients studied and control subjects.

With regard to ventilation capacity the mean intensity of the asthma during the five years almost immediately preceding the test of this function appeared to have greater influence on the result than the over all degree of severity since the onset of this disease. On the other hand the increase in the ratio of the residual volume (RV) to the total lung capacity (TLC) a measure of hyperinflation, appears to depend mainly on the degree of severity of the disease as estimated for the whole period since it was first manifested. It is plausible that the reduction in ventilation capacity — which can be demonstrated in free intervals — is to some extent of a reversible nature. Reversibility appeared to be valid to a lesser extent for the state of hyperinflation. It is supposed that hyperinflation — although an at least partly irreversible sign in the present series — does not necessarily imply a state of emphysema (defined as a condition complicated by alveolar wall destruction)

A significant correlation was found for the patients in the present series between ventilation capacity and physical work capacity The partial correlation between physical work capacity and the estimated degree of severity of the bronchial asthma — when the influence of the reduction of ventilation capacity has been eliminated — was low This indicates that the influence of the bronchial asthma on the physical work capacity was exerted to the greatest part via the influence on the ventilation capacity The relationship between the ratio RV/TLC and physical work capacity was found to be of no appreciable importance.

A study was made with the purpose of ascertaining whether certain factors occurring in the case history of these patients had any statistically significant influence on function variables such as the pressure in the pulmonary artery arterial oxygen saturation etc The results obtained indicate that the effects of the disease on the pulmonary circulation — demonstrable during symptom free intervals — may be to some extent reversible, i e. they diminish when the disease becomes less active.

In comparison with the control material the patients in the asthma series showed on an average a relatively strong tendency to an increase in the ratio of the residual volume to the total lung capacity Since there was no pulmonary hypertension under ordinary resting conditions it may be probable that the increased ratio RV/TLC was not caused by extensive destructive pulmonary emphysema It is more likely that this increase was due only to a less complicated state of hyperinflation. A reasonable conclusion therefore is that bronchial asthma — even if it has previously been intensive for long periods — does not regularly lead to pulmonary hypertension if the disease is not complicated by other conditions which lead to destruction of lung tissue.

In hospital records of patients with a long history of bronchial asthma which during certain periods has been very intensive it is not unusual to find a suggestion and sometimes a definite statement, that so-called cor pulmonale is present. In all of the patients in the present series who underwent heart catheterization however the resting pressure in the lesser circulation lay within normal values.

No statistically significant excess mortality was shown in the present study Disregarding the five patients who were considered to have died by direct reason of an asthmatic attack, the observed number of deaths in this series was in agreement with the predicted. It is reasonable to conclude, therefore that uncomplicated bronchial asthma — if death from an acute attack as such can be prevented — does not regularly involve any noteworthy risk of excess mortality by reason of secondary effects on the cardiac and pulmonary functions and the pulmonary circulation.

The results of the socio-medical investigations on this group of patients indicated that their degree of social adaptation was, on an average good. When — for some indicators studied — the asthma series was classified into groups according to the estimated degree of severity of the disease no noteworthy difference was observed between these groups. On assessing the socio-medical consequences of this disease, however several factors should be taken into consideration for instance the relatively large number of days of incapacity due to asthma and days with symptoms and the fact that approximately every fifth patient in this series had received an invalid pension solely or mainly because of asthma another important factor is the large demand on hospital care and medical attendance It was clear that measures of support on the part of the community and private individuals

were of great importance to these patients. The relatively bright picture of the social adaptation of this group, shown in most respects by the results obtained, was probably due essentially to the fact that in practically all of these patients the course of the disease was intermittent. For the twenty year period preceding the present investigation the labour market was on the whole favourable, which must have been of great importance for persons handicapped by bronchial asthma with regard to obtaining work and earning a living. The results of the investigation also appear to indicate that in many of the patients the disease demanded an assiduous manner of living. The fact that for certain of the indicators of social adaptation studied here the present group of patients exhibited a status that to some extent appeared to deviate favourably from that of the control subjects may partly be explained by such a manner of living.

Other important probable reasons for the satisfactory social situation found on the whole in these patients, apart from those mentioned previously, are that the effects of the disease on the respiratory and circulatory functions appeared to be at least in some respects, reversible and also that the differences from ordinary respiratory and circulatory conditions, found in this study were not of such a type and degree as to cause permanent severe disablement. The favourable course of the disease — either spontaneous or due to therapy — found in the majority of the patients during the last few years preceding the investigation had made it possible for many to regain the loss in social situation that may previously have been caused by periods of incapacity of varying lengths. The results of the investigation also indicate that the patients had been able to utilize the improvement in their health condition for social stabilization.

STATISTICAL METHODS

Notation

Mean $\bar{x} = \frac{\sum x_i}{n}$ where x_i

denotes the i th recorded value in a series of n values.

Relative frequency

$$p = \frac{\text{number of incidences}}{n}$$

Standard deviation

$$SD = \sqrt{\frac{\sum(x_i-\bar{x})}{n-1}}$$

Standard error of the mean (SE)

$$SE = \frac{SD}{\sqrt{n}}$$

in comparison between two groups

$$SE_{\bar{x}-\bar{y}} = \sqrt{\frac{\sum(x_i-\bar{x})^2}{(n-1)\,n} + \frac{\sum(y_i-\bar{y})^2}{(n_y-1)\,n_y}}$$

Standard error of a relative freq

$$SE = \sqrt{\frac{p(1-p)}{n}}$$

standard error of difference between two relative frequencies or percentages

$$SE_{p_1-p_2} = \sqrt{\frac{p_1(1-p_1)}{n_1} + \frac{p_2(1-p_2)}{n_2}}$$

Standard weighted percentages

$$\sum W_j P_j$$

where P_j is the percentage in the j th sub-group

W_j *is the weight of the j'th* sub-group and

$$\sum W = 1$$

Weighted percentages are applied when comparing groups differing in age distributions The weights, W_j are chosen to correspond approximately to the number of subjects in the respective sub-groups.

Multiple regression analysis

Linear regression analysis has been performed

A variate, y is considered a linear function of k variables x_1 x_2 ... x_k i e.

$$y = a + b_1 x_1 + b_2 x_2 \quad + b_k x_k$$

We calculate the coefficients b_1 b_2 ... and b_k according to the least square principle (*cf* Snedecor 1956, and Hald, 1948)

Calculations of expected mortality

In order to compare the observed and expected mortalities, the annual death risks for each individual were first estimated and in the ordinary way consideration was given to sex, age, decade and length of exposure. For this purpose, life tables (Mortality and Length of Life tables) for Sweden were used. For each individual the annual death risks were summated for all the years he was exposed, i e. between admission and death or admission and withdrawal from the investigation (1961—62) This sum was called e If the e_i values are summated for a patient group the total obtained Σe_i = expected number dead The expected and observed numbers are then compared. For analysis of significance the differences $o_i - e$ can be obtained where o = 1 if the i th person died and = 0 if he had not died. If these

differences are called x_i then SD and SE can be calculated as given above.

Significance tests

The following significance tests were used

1 In percentage differences χ^2 test usually with one degree of freedom
2 In mean differences

$$CR = \frac{\bar{x} - \bar{y}}{SE_{\bar{x}-\bar{y}}}$$

which approximately follows the standardized normal distribution (or t test if $n < 30$)

3 In differences between standard weighted percentages

$$\sum W_j (P_{xj} - P_{yj})$$

a normally distributed critical ratio is produced

$$CR = \frac{\sum W_j (P_{xj} - P_{yj})}{\sqrt{\sum W_j^2 \frac{n_{xj}P_{xj} + n_{yj}P_{yj}}{n_{xj} + n_{yj}}\left(100 - \frac{n_{xj}P_{xj} + n_{yj}P_{yj}}{n_{xj} + n_{yj}}\right)\left(\frac{1}{n_{xj}} + \frac{1}{n_{yj}}\right)}}$$

where n_{xj} and n_{yj} correspond to the number of subjects in the compared sub-groups.

4 On comparing the observed and the expected number the Poisson distribution has been used as an approximation if the expected number was small
5 On comparing the observed and expected mortality the individual differences were first calculated and then

$$CR = \frac{\sum x_i}{n\,SE}$$

where n is the number of individuals and $x = o - e$

The same formula is used when comparing expected and observed demographic and social characteristics (married, over-crowded etc.)

6 In analysis of mean rank, the following approximately normally distributed CR was produced

$$CR = \frac{\text{mean rank } \% - 50\,\%}{\sqrt{\frac{1}{12\,n}}}$$

7 In regression and correlation analysis tests of coefficients as in Snedecor (1956)

In the significance analyses the patients constituted the unit.

For references on significance tests see Snedecor (1956) and Hald (1948)

Significance levels

Analyses of significance have been made chiefly in conjunction with the tables. The term significant is used in accordance with the following convention. If an observed difference between two percentages (or two means) is of such magnitude that the probability P of obtaining a difference at least equal to the observed value is greater than 0.05 (where the null hypothesis is assumed to hold) the observed difference is said to be *non-significant*

If $0.01 < P \leq 0.05$ the difference is said to be *(almost) significant* and is denoted by

If $0.001 < P \leq 0.01$ the difference is said to be *significant* and is denoted by

If $0.001 \geq P$ the difference is said to be *(highly) significant* and is denoted by

Appendix 2

Case reports of patients with a chronic disease in addition to the bronchial asthma

Those patients who had or had had another chronic disease — *in addition* to the bronchial asthma — have been divided into two groups as below

Group I

Group I comprises patients who were excluded from the general analyses because they had another chronic disease — in addition to the bronchial asthma — which was judged to have had a decisive influence on their life in *general state of health and functions*

Case No 12. A. J Male born 1926. Chronic bronchitis+healed pulmonary tuberculosis. Divorced, no children, photographer Chronic bronchitis since 1932. Only a few years after the onset of bronchitis this was gradually *complicated by symptoms of bronchial asthma.* About 1934 sanatorium treatment for 18 months for bilateral pulmonary tuberculosis. Repeated follow up examinations showed nothing of note. Right middle lobectomy 1939 *for bronchiectasis.*

Excluded from the analyses mainly because of chronic bronchitis.

Case No 45. K. K Femal born 1911 Diabetes mellitus+healed pulmonary tuberculosis. *Married 1 child, housewife Onset of* bronchial asthma 1943 in which year diabetes mellitus was also manifested, for which continuous insulin treatment had si ce been given Had been receiving an invalid pension since *1945 because of asthma. Since the middle* of the 1940 s had had follow up examinations f r suspected tuberculous lung changes. Chest X ray at the time of the present investigatio showed left apical changes in the f rm of *patches nd treaks,* and on the right ld small treaky opacities in I 1—I 2.

Ex l led mainly beca se of *diabetes mel litus.*

Case No 66. G S M le born 1904 Chronic bronchitis. Married, 1 child, ex-paint r Chronic bronchitis ince 1932. *Only a few* years after th onset of chronic bronchitis, this wa gradually complicated by symptoms of bronchial asthma. Recei ed invalid pension I 1933 because of bronchial asthma.

Excluded mainly because of chronic bronchitis.

Case No 68. F J Female born 1903. Pulmonary sarcoidosis. Unmarried, nulliparous, charwoman. Onset of bronchial asthma 1946. *Admitted to hospital 1950* and *1957* for investigation of lung changes which were suspected to be tuberculous but which were later found to be probably sarcoidosis. Mantoux test had never shown a positive res lt. Chest *X-ray at the time of the present investigation* revealed large shrunken opacities in th upper regions of both lungs with pleural thickening surrounding the upper parts of the lungs in particular but also the bases, where this was *most pronounced on the right side*

Excluded because of pulmonary sarcoidosis.

Case No. 70 S E. Mal born 1903. Healed pulmonary tuberculosis (?) Married, 1 child, typographer Onset of bronchial sthma 1944. *Admitted to hospital at the beginning f the* 1950 s fo in estigation of tuberculous-like lung changes. No tubercle bacilli shown Chest X ray at the time of the present investigation revealed streaky and patchy to great part *calcified, parenchymal* us pacities in both apical fi lds. Bronchography showed an irregular shaped *cavity about the size of a* grape in the left lower l be suspected to be tuberculous.

*Excluded because of extensive roentgenolog*ical lung changes, probably tubercul us.

Case No 7- *E. L. Female born* 1902. Healed pulmonary tuberculosis. M rried, nulliparous, housewife. Onset *f bronchial a thma* 1944. Pulmonary tuberculosis 1948 (left sided lung process with ca ity tubercle bacilli in *p tum) f r* which sanatori m treatment was given f r 13 months. (1950 recurrence; sp t m nce more *co tained t bercle* bacilli) 1918

received invalid pension because of pulmonary tuberculosis. Chest X-ray t the time f the present investigatio revealed bilateral tuber culous changes with shrinkage of the left upper lobe in hich region cystif rm rarefac tions were seen. Pleural thickening in the left apex. Small streaky opacities in the right upper lobe Upward displacement f the left hilus

Excluded because of healed pulmonary tuberculosis.

Case No. 82. I. A Female, born 1901. Mitral valv insufficiency M rried, 1 child, house wif Onset of bronchial sthma in earl childhood. Mitral insufficiency diagnosed in 1952. Diagnosi confirmed by angiography from the left entricle Somewhat raised lung capillary pressure. ECG howed igns of left entricula hypertrophy

Excluded because of mitral i nsuf ficiency

Case No. 101 L. L. Female, born 1897 Previous cardiac inf rction. Married, 1 child. housewife. Onset of bronchial sthma 1943. Probable cardia infarct 1954 in which year the received nvalid pension been se of cardiac arteriosclerosis. ECG revealed partial left bundle branch block. Some enlargement of left entricle seen on roentgenological exa mination

During recent years had had increasing symptoms f left cardiac failure

Excluded because f left cardiac failure.

Case No 106 K W Male born 1896 Chronic myocarditis. Married, 1 child, fore man Onset of bronchial asthma 1918. Auricul ar fibrillation, probably due t myocarditis, diagnosed 1946. Marked general enlargement of the heart found on X-ray ECG revealed ricular fibrillatio and generally low QRS amplitudes

Excluded because of chronic myocarditis.

Case No 111 F L. Female born 1893. Mitral valve insufficiency + angina pectoris. Married, all parous, housewif Onset of bronchial asthma 1908. Moderate arterial hyp ertension know for bout 10 years. In 1952 marked physical findings indicated organic disease of the heart, which since this time had been generally enl rged For the la t tw or three years the patient had had interm ttent precordial pain of ngina pectoris type

E luded because of mitral h insuffici ency

Case No. 112. H. A. Male born 1895. Cer bro-vascular lesion. Widower 4 children, ex f rmer Onset of bronchial sthma 1930 Rec eived an invalid pension in 1948 because of cerebral thrombosis. Diabetes mellitus since 1942, treated with insulin since Cerebro-vasc ula lesio 1946.

Excluded because of cerebro- scula lesion nd diabetes mellitus.

Case N 118. J F M le, born 1895. Chronic *rheumatoid arthritis*. M rried, 8 children previously cattleman. Onset of bronchial asth ma 1911. Received inv lid pension 1947 be cause of bronchial asthma. 1930 manifestation of rheumatoid rthritis, which became grad ually worse inv l ing mainly the joint of the hand nd feet. From bout 1935 thi latter cond tion rapidly progressed.

Excluded because of chronic rheumatoid rthritis.

To summari.e the following diseases influenced the function and general situation of the 12 patients to such a great extent that it was considered that they should be excluded from the general analyses of the investigation results healed pulmonary tuberculosis (2 patients) organic disease of the heart (2 patients) chronic bronchitis (patients) pulmonary sarcoidosis (1 patient) diabetes mellitus (1 patient) cerebro-vascular lesion (1 patient) previous cardiac infarction (1 patient) chronic myocarditis (1 patient) and chronic rheumatoid arthritis (1 pat ient)

For the tw patie t (case Nos. 12 nd 66) ith *hronic bronchitis*, it cannot be tated th certainty whether this wa primary *condition* or occurred as complication of bronchial asthma. Both were stated that they had had chronic cough since the beginning of the 1930's, and that they had no thma symptom before this time Both, however stated that more marked these symptoms

did not appear until a few years after the onset of chronic bronchitis. Since it could not be precluded with certainty that the chronic bronchitis occurred as a complication of the bronchial asthma some complementary data are given below for these two patients The figures given first are those for the younger of the two men

PWC$_{130}$ 23 /$\!_{\circ}$ of predicted value, —
PWC$_{max}$ 25 % of predicted value, —
FEV$_{1.0}$ 10 /$\!_{\circ}$ of predicted value, 23 %
FEV% 51 % of predicted value, 51 %
MVV$_{40}$ 21 % of predicted value, 26 %
RV/TLC 238 % of predicted value, 209 %.
mean pressure in pulmonary artery at rest 12 mm Hg 18 mm Hg
mean pressure in pulmonary artery during exercise (300 and 150 kpm/min respectively) 35 mm Hg 30 mm Hg
cardiac output 5 1 litres/min, 5.6 litres/min.
stroke volume 52 ml, 68 ml.
a—v O_2 difference 56 ml/min, 47 ml/min.
Housing standard (according to the ranking method, see page 31) 2 0 /$\!_{\circ}$, 15.9 /$\!_{\circ}$.
Income at the end of 1963 both were receiving an invalid pension

To summarize it may be said of these two patients that in relation t both the asthma series and the control material th y exhibited on the whole a relatively unfavourable status with regard to the abo e mentioned indices, with the ception however of the values obtained in connection with heart catheterization, where both the pressure and flow conditions were found to lie within normal limits.

Group 2

Group 2 comprises patients who — in addition to the bronchial asthma — had or had had another disease or suspected signs of another disease. It was not considered in these patients, however that this supervening disease was of such a type or degree as to have had a decisive influence on their function or general situation The patients in this group were therefore included in the general analyses of the results.

Case No 25. G I Female, born 1918. Benign hypertension. Married, 2 children, telephonist. Onset of bronchial asthma 1938. Arterial hypertension since 1950 but there had been long periods when the blood press re was practically normal. During heart catheterization at the present investigations the blood pressure was found to be $\frac{140}{70}$ 95 when no anti hypertensive treatment was being given. At the time of these in estigations no abnormal signs indicating vascular complications were observed. Neither X ray examination nor ECG revealed any cardiac abnormalities

Case No. 34 L. S. Femal born 1914 Previous cardiac infarction? Married, 3 children, housewife. Onset of bronchial asthma 1950. Admitted to hospital 1953 for observation of suspected cardiac infarct No further cardiac symptoms. At the time f the present in estigation neither X ray nor ECG revealed any pathological cardiac condition

Case No 39. M S. Female born 1913. Hypertension+hypothyroidism. Married, n llpar ous hairdresser Onset of bronchial asthma 1933. Arterial hypertension since about 1955. During the years the blood pressure had ar ied greatly and during heart cath terization at the present investigation a value of $\frac{190}{100}$ 135 was found in the absence of anti hypertensive treatm nt. X ray examination revealed no cardiac abnormality and the ECG picture was not typical of left ventricular hypertrophy At the time of the present investigation no abnormal signs f vascular complications were observed and neith r were there any signs of hypothyroidism f r which the patient wa treated at the end of the 1950 s.

Cas No. 40. M. N Female, born 1912. Chronic rheumatoid arthritis. Widow 1 child, charwoman. Onset f bronchial a thma 1944 Rheumat id arthritis 1956, f llowed by slight residual symptoms i the hand and foot joints.

Case No 44 E. L. M le born 1911 Healed tuberculous infection f tracheobronchial lymph g ands. M rried 1 child fact ry w rk

er. Onset of bronchial asthma 1931. Sanatorium treatment 1927 for suspected tuberculosis. Chest X-ray showed incipient tuberculous changes in hilus. Tubercle bacilli not shown. Patient discharged as healthy and no follow up examinations made. Chest X-ray at the time of the present investigation revealed nothing abnormal.

Case No. 68. R. E. Male, born 1906. Previous poliomyelitis. Single, no children, solicitor's clerk. Onset of bronchial asthma 1932. Poliomyelitis at the age of 1 year mainly affecting the right leg, which was now slightly shortened with some muscular atrophy. This patient was not included in the calculations of the physical work capacity in the present investigation.

Case No. 78. S. S. Male, born 1902. Previous cardiac infarction. Unmarried. Onset of bronchial asthma 1912. Had received invalid pension for bronchial asthma since 1928. Admitted to hospital 1933 for suspected cardiac infarct. The ECG picture at the time of the present investigation revealed nothing definitely pathological. No definite ECG signs of coronary insufficiency were observed during the work test, and there were no definite symptoms of angina pectoris either during or after this test. X-ray examination revealed no cardiac abnormality.

Case No. 87. T. J. Female, born 1899. Right knee deformed by arthritis. Married, 2 children, housewife. Onset of bronchial asthma 1914. Arthritis of right knee since about 1933, and the patient was therefore not included in the calculations of the physical work capacity.

Case No. 93. C. C. Female, born 1898. Chronic rheumatoid arthritis. Married, 2 children, housewife. Onset of bronchial asthma 1940. Rheumatoid arthritis since 1934, which had slowly progressed, involving mainly the knee and hand joints. The changes were of mild type.

Case No. 98. J. K. Female, born 1898. Mild Parkinson's disease. Onset of bronchial asthma 1933. Mild symptoms of Parkinson's disease since the end of 1939.

Case No. 99. P. G. Female, born 1898. Pernicious anaemia with previous episodes of mild myelopathia + mild diabetes mellitus. Married, 1 child, housewife. Onset of bronchial asthma 1929. After ... B₁₂ test ... indicating pernicious myelopathy were obtained in 1936. At about the same time mild diabetes mellitus was manifested, but this did not require any special therapy or diet, and the blood sugar values had remained within normal limits.

Case No. 100. T. M. Female, born 1897. Anaemia and high sedimentation rate of unknown cause. Married, 1 child, housewife. Onset of bronchial asthma 1938. On admission to hospital for the present investigations, moderate anaemia (11.1 g %) and high sedimentation rate (131 mm) were observed. Examinations for possible malignancy were made, with negative results. This patient is represented in the result analyses by those values obtained at the out-patient stage of these investigations, at which time — 7 months before admission to hospital — both the Hb content and sedimentation rate were normal.

Case No. 101. H. P. Female, born 1897. Previous manio-depressive psychosis. Widow, 1 child. Onset of bronchial asthma 1932. Treated in a mental hospital on a few occasions during the 1930's for manio-depressive psychosis. Operated on in 1927 and 1928 for thyrotoxicosis.

Case No. 107. E. L. Female, born 1896. Healed pulmonary tuberculosis. Unmarried, nulliparous, ex-nurse. Onset of bronchial asthma 1906. In 1917 chest X-ray revealed patch on the right lung, diagnosed as tuberculosis. Sanatorium treatment given for about 6 months. Chest X-ray at the time of the present investigation showed streaky and patchy right apical opacities, otherwise nothing abnormal.

Case No. 115. N. N. Male, born 1893. Gastroduodenal resection + resection of colon. Married, 2 children, previously mill fitter. Onset of bronchial asthma 1940. Gastro-duodenal resection + resection of colon in 1934 for large cancer of stomach infiltrating into the mesocolon. Subsequent course free of complications.

Case No. 120. C. S. Male, born 1891. Healed pulmonary tuberculosis. Widower, 2 children, previously wood-worker. Onset of bronchial asthma 1948. In about 1925 lung changes were seen at both apices, suspected to be tuberculous. Tubercle bacilli never shown. Chest X-

ray at the time of the present investigation *showed occasional calcifications in both apices* and a few calcifications in the middle part of the right lung. No signs of active tuberculosis.

Patients with a relatively large deviation from normal values with regard to basal metabolic rate and hemoglobin content

Apart from the conditions present in the patients of groups 1 and 2, described above, *no other signs of supervening diseases were* observed during these investigations. A brief report will be made below however of those cases where relatively large deviations from normal values were found for basal metabolic rate and hemoglobin concentration.

A *basal metabolic* rate exceeding +20 % of the predicted value (prediction according to sex, age, height and body weight, Harris and Benedict, 1919) was recorded for the following patients: Case No. 97 (21 %), No. 65 (22 %), No. 20 (22 %), No. 78 (26 %), No. 115 (28 %), No. 110 (31 %), No. 113 (31 %), No. 81 (36 %), No. 98 (37 %), No. 116 (45 %), No. 114 (47 %). A basal metabolic rate of less than — 20 % of the predicted value was noted for case No. 43 (—23 %).

In none of these patients or in any of the other patients in the asthma series was there any reason to suspect current thyrotoxicosis or myxoedema.

During the investigations the following women were found to have a *hemoglobin concentration* of less than 11.5 gram/100 ml blood: *Case No. 30 (11.3 g %), No. 29 (10.0 g %) and* No. 57 (10.5 g %).

One of the men, case No. 72, was found to have a hemoglobin concentration of less than 12.5 g/100 ml blood, i.e. 11.7 g %.

No signs or symptoms *indicating* cardiac or pulmonary or other diseases, apart from those mentioned, were found in this series of patients.

Key to the ward case records

Case No.	Case record No.	Case No.	Case record No.	Case No.	Case record No.
1	2136/48	44	718/62	87	[illegible]/48
2	2031/62	45	1110/62	88	805/62
3	714/47	46	367/62	89	2212/62
4	331/63	47	488/60	90	344/62
	3732/62	48	355/46	91	1218/63
6	2161/62	49	141/47	92	1808/63
7	2302/63	50	322/63	93	1121/63
8	2341/62	51	1971/63	94	2180/63
9	2048/63	52	163/63	95	2130/62
10	263/54	53	1808/62	96	2179/62
11	2234/62	54	482/62	97	912/40
12	1962/63	55	1107/62	98	1187/53
13	2670/62	56	2078/62	99	1103/62
14	2530/62	57	384/62	100	863/48
15	1991/62	58	827/63	101	1133/63
16	1193/63	59	478/62	102	963/62
17	2037/62	60	110/63	103	2561/62
18	910/62	61	1229/62	104	1311/62
19	517/62	62	108/62	105	1414/62
20	431/62	63	333/63	106	2324/62
21	2112/62	64	2382/63	107	782/62
22	872/62	65	2792/62	108	788/63
23	2164/62	66	330/63	109	1981/62
24	2612/39	67	2185/62	110	1061/62
25	[illegible]/63	68	[illegible]/62	111	2090/62
26	398/62	69	[illegible]/62	112	2003/61
27	2131/62	70	[illegible]/62	113	2152/62
28	2707/63	71	367/62	114	1228/62
29	742/63	72	2961/62	115	1302/62
30	772/63	73	200/63	116	1888/62
31	775/62	74	[illegible]/62	117	2717/63
32	312/62	75	1678/63	118	1802/63
33	990/62	76	354/62	119	[illegible]/63
34	183/62	77	121/63	120	1062/62
35	[illegible]/59	78	327/63	121	1905/62
36	718/62	79	480/62	122	[illegible]/58
37	1159/48	80	770/62	123	1860/62
38	218/15	81	378/62	124	2353/62
39	164/63	82	1464/62	125	321/50
40	1132/63	83	2858/63	126	2037/63
41	1186/62	84	2538/62	127	1202/62
42	243/62	85	789/49	128	992/62
43	616/64	86	875/62		

[illegible] otherwise stated the numbers ref[illegible] records of the Medical Clinic, University [illegible]ital, Uppsala.

[illegible] of the Ear, Nose and Throat [illegible]

[illegible] case record of the Chest Clinic, University Hospital, Uppsala.

[illegible] case record of the Samaritan Hospital, Uppsala.

REFERENCES

AAS, K. Prognosis for asthmatic children *Acta Paed Scand* 140: 87 1963.

ABBOTT O A HOPKINS, W A., VAN FLAIT W E. and ROBINSON S. A new approach to pulmonary emphysema. *Thorax* 8 116, 1953

ALEXANDER H L.: Proceedings of the third *international congress of allergology* p. 109 Flammarion, Paris 1958.

AMERICAN THORACIC SOCIETY *Chronic bronchitis, asthma and pulmonary emphysema. Am r Rev Resp Dis* 85 762 1962.

AREIKOG N H and BACKLUND L. Estimation of basal oxygen uptak. *Nord Med* 65 734 1961

ASK LEMARK, E. Life death and diseas in Sweden. Almqvist & Wiksell Stockholm, 19..

ASMUSSEN E. and NIELSEN M.: Cardiac output in rest and work at low and high o ygen pressures. *Acta Ph y iol Scand* 35 3 1955.

ÅSTRAND, IRMA Aerobi work capacity in men and women with special ref rence to age *Acta Physiol Scand* S ppl 109 1960

ÅSTRAND, P-O Experimental studies of phys ical working capacity in relation to sex and age Ejnar M nksga rd, Copenhagen, 1952

BALDWIN ELEANOR DE F Bro chial asthma *Amer J Med* 1: 180 1946.

BALDWIN ELEANOR DE F COURNAND A and DICKINSON W B Pulmonary in ufficiency *Med* 28 201 1949

BARKER, S. B. nd SUMMERSON W H The colorimetric det rmination of lacti acid in bi logical material. *J Biol Chem* 138: 535 1941

BATES, D V KNOTT J M S nd CHRISTIE, R V Respiratory function i emphysema in relation t prognosis. *Quart J Med* 25 13 1956.

BEALE, H D FOWLER, W S. and COMROE, J H Pulmonary function studies in 20 asthmatic patients in the symptom free interval. *J All* 23 1 1952.

BEHNKE, R. H WILLIAMS, J F and WHITE, D H The effect of acetylcholine i fusion upon cardiac dynamics in patients with pulmonary emphysema *Amer Rev Resp Dis* 87 57 1963.

BENSTEN B G Forekomst og invaliditet ved a tma — bronkitt — emfysem. *Tidskr for Den N rske laegeforening* 16 102 1962

BERGLUND E., BIRATH, G BJURE, J GRIMBY G KJELLMER, I SANDQVIST L. and SODERHOLM, B Spirometric tudies in n rmal subjects. I Forced expirograms in subjects between 7 nd 70 years of age *Acta Med Scand* 173 185 1963.

BERNSTEIN L. D'SILVA, J L. and MENDEL D The effect of the rate of breathing on th ma imum breathing capacity determined with a new spirometer *Th rax* 7 255, 1952.

BEVEGÅRD S HOLMGREN A nd JONSSON B The effect of body postur o the circulat ion at rest and during e ercise with special reference to th i fluence n the trok olume *Acta Physiol Scand* 49 279 1960.

BIRATH, G. KJELLMER, I nd SANDQVIST L. Spirometri studies in normal subjects. II. Ventilatory capacity test in ad lts. *Act Med Scand* 173 193 1963.

BOLT W VALENTIN H, and TIETZ, N Drucke in d r Pulmonalarteri Herzminuten V lumen und Atmu g bei ak ter re pirato rischer Hypoxie nt p echend Höhen l is zu 4000 und 6000 M f r bei ältere Per sonen. *Arch Kreisl F rsch* 2 18, 1957

BORDEN, C. W., WILSON, R. H., EBERT, R. V. and WELLS, H. S. Pulmonary hypertension in chronic pulmonary emphysema. *Amer J Med* 8 701, 1950.

BOWER, G. Deaths and illness from bronchitis emphysema and asthma. *Amer Rev Resp Dis* 83 894 1961

BRUCE, T., DAHLSTROM, G. and UGGLA, L.-G. Sociomedical studies of tuberculous patients from Stockholm. The social structure of the material. *Acta Tuberc Scand Suppl* 50 9 1960.

CARDELL, B. S. and BRUCE PEARSON, R. S. Death in asthmatics. *Thorax* 14 341, 1959

CIBA GUEST SYMPOSIUM. Terminology definitions and classification of chronic pulmonary emphysema and related conditions. *Thorax* 14 286,1959

CLAUSEN, O. Asthma. Norge. *Nord Med* 37 [illegible] 1948.

COTES, J. E., PISA, Z. and THOMAS, A. J. Effect of breathing oxygen upon cardiac output, heart rate, ventilation, systemic and pulmonary blood pressure in patients with chronic lung disease. *Clin Sci* 25 305, 1963.

DAHLBERG, G. Sinnessjuka. fruktsamhet. *Nord Med* 6 [illegible] 1942

DAHLSTROM, G. Studies of the physical working capacity in pulmonary tuberculosis. *Nord Med* 53 417 1955.

DAHLSTROM, G. Physical working capacity and exercise electrocardiogram in pulmonary diseases. *Acta Tuberc Scand* 34 [illegible], 1957

DEXTER, L., WHITTENBERGER, J. L., GORLIN, R., LEWIS, B. M., HAYNES, F. W. and SPIEGL, R. J. *Trans Assoc Amer Physic* 64 226, 1951

DONALD, K. W., BISHOP, J. M. and WADE, O. L. A study of minute-to-minute changes of arterio-venous oxygen content difference, oxygen uptake and cardiac output and rate of achievement of a steady state during exercise in rheumatic heart disease. *J Clin Invest* 33 1146, 1954

EARLE, B. V. Fatal bronchial asthma. *Thorax* 8 195 1953

ESSEN-MÖLLER, E. Untersuchungen über die Fruchtbarkeit gewisser Gruppen von Geisteskranken. Schizophrenen, Manisch-Depressiven und Epileptikern. *Acta Psychiat Suppl* 8 1935

FAGERBERG, E. Studies in bronchial asthma. Acta Allerg XI 327 1957

FISHMAN, A. P., McCLEMENT, J., HIMMELSTEIN, A. and COURNAND, A. Effect of acute anoxia on the circulation and respiration in patients with chronic pulmonary disease studied during the steady state. *J Clin Invest* 31 770, 1952.

FISHMAN, A. P., FRITTS, H. W. and COURNAND, A. Effects of acute hypoxia and exercise on the pulmonary circulation. *Circulation* 22 204 1960

—Effect of breathing carbon dioxide upon the pulmonary circulation. *Circulation* 22 220 1960

FLETCHER, C. M., HUGH-JONES, P., McNICOL, M. W. and PRIDE, N. B. The diagnosis of pulmonary emphysema in the presence of chronic bronchitis. *Quart J Med* 32 33, 1963.

FOWLER, N. O., WESTCOTT, R. N., SCOTT, R. C. and HESS, EMIL. The cardiac output in chronic cor pulmonale. *Circulation* 6 888, 1952.

FRITTS, H. W., HARRIS, P., CLAUSS, R. H., ODELL, J. E. and COURNAND, A. The effect of acetylcholine on the human pulmonary circulation under normal and hypoxic conditions. *J Clin Invest* 37 99 1958.

GAENSLER, E. A. Evaluation of pulmonary function. Results in chronic obstructive lung disease. *Ann Rev Med* 13 319 1962

GARBY, L. and VUILLE, J.-C. The amount of trapped plasma in a high speed micro-capillary hematocrit centrifuge. *Scand J Clin & Lab Invest* 13 642, 1961.

GLOOR, F. Zur Pathologie des Asthma bronchiale. *Virchows Arch Path Anat* 323 189 1934.

GOUGH, J. Discussion on the diagnosis of pulmonary emphysema. *Proc Roy Soc Med* 45 576 1952.

GRAM, H. C. Asthma bronchiale. *Nord Med Tid*skr 3 616, 1931

GRANATH, A., JONSSON, B. and STRANDELL, T. Studies on the central circulation at rest and during exercise in the supine and sitting body position in old men. *Acta Med Scand* 169 125, 1961.

GRIMBY, G. and SÖDERHOLM, B. Spirometric

WALKUP H E and CONNOLLY ELEANOR C.: The dimensions of the chronic respiratory disease problem *Amer J Publ Health* Suppl 53 1963

WESTCOTT R N FOWLER, N O SCOTT R C., HAUENSTEIN V D and MCGUIRE, J Anoxia and human pulmonary vascular resistance *J Clin Invest* 30: 957 1951

WIKLANDER, O: Blood volume determinations in surgical practice. *Acta Chir Scand* Suppl 208, 1956

WILLIAMS, D A Social importance of allergic diseases. Premier Congrès Internationel d'Allergie Zürich 1951 p. 42 Karger Basel.

WILLIAMS, D A Deaths from asthma in England and Wales. *Thorax* 8 137 1953.

WILLIAMS, D A. and LEOPOLD J G.: Death from bronchial asthma III Congr International d'Allergologie Paris 1958. *Acta All* 14 83 1959

WHITAKER, W: Pulmonary hypertension in congestive heart failure complicating chronic lung disease *Quart J Med* 23 57 1954

YU P N., LOVEJOY F W JOOS, H A., NYE, R. E. and MCCANN W S Studies of pulmonary hypertension. *J Clin Invest* 32 130 1953.

ACKNOWLEDGEMENTS

This study which was carried out during the years 1961–64 was originally suggested by Professor Erik Ask-Upmark, head of the Department of Internal Medicine University Hospital, Uppsala under whose guidance I have had the privilege of working. I am greatly indebted to him for his valuable advice and the continuous interest which he has shown in my work. Professor Ask-Upmark supervised the patients in this study and his extensive knowledge of bronchial asthma has been inestimable to me.

Professor Ragnar Berfenstam—under whom I am working at present—head of the Department of Social Medicine, University Hospital, and Professor Gunnar Strom, head of the Department of Clinical Physiology University Hospital, who generously placed their departments at my disposal—both participated in the original planning of this study. Their great interest, encouraging guidance and constructive criticism throughout the course of the investigation, have been of vital importance and I would like to express the sincere gratitude and appreciation which I feel toward them.

Docent Ivar Werner has given me constant support from the beginning of the work. His great interest, helpful and stimulating advice during the course of the study have been of great value to me and I wish to express my sincere thanks.

I would also like to express my appreciation to Docent Gunnar Eklund for his great help with the statistical analyses. I thank him for his unfailing interest in my work and for innumerable fruitful discussions.

Professor Erik Knutsson, head of the Department of Diagnostic Radiology University Hospital, and Docent Herman Lodin generously placed their laboratory at my disposal and gave me valuable help in judging the X-ray films. All heart volumes were calculated with the aid of Doctor Björn Carlsson to whom I express my thanks.

Professor Erik Hedvall, former head of the Chest Department University Hospital, Professor Arne Sjöberg, head of the Department of Otolaryngology University Hospital, Docent Runar Brenning, head of the Medical Department of the Samaritan Hospital, Uppsala, and Doctor Erik Fagerberg, consultant allergist at University Hospital, have kindly placed at my disposal information about patients who are or have been under their supervision.

I should like to acknowledge the great help which I have received from the staff of the Departments of Internal Medicine Clinical Physiology Social Medicine and Diagnostic Radiology and the Chest Clinic, University Hospital.

I express my thanks to Miss Birgitta Karendal for invaluable help in the analyses of blood and gas samples and also to Mrs Frauke Graf for help with figure drawings.

The work has been translated by Mrs Maud Marsden. I wish to thank her for helpful co-operation.

I would like to express my appreciation to the Swedish National Association for Heart and Chest Diseases and to the Medical Faculty of the University of Uppsala for the generous grants made to defray the costs of my investigation.

To all others who have in various ways made it possible for me to carry out this work, I extend my sincere thanks.

Lars Irnell

ACTA MEDICA SCANDINAVICA

SUPPLEMENTUM 418

EXPERIMENTAL URAEMIC PULMONARY OEDEMA

including criteria for pulmonary oedema
in uraemic rabbits, the role of uraemia and overhydration,
and a literary survey on the problems
of uraemic pulmonary oedema (fluid-retention lung, etc.)

by

BENGT LINDQVIST

Accompanies Vol. 176

LUND 1964

ACTA MEDICA SCANDINAVICA

SUPPLEMENTUM 418

EXPERIMENTAL URAEMIC PULMONARY OEDEMA

including: criteria for pulmonary oedema
in anuric rabbits, the role of uraemia and overhydration,
and a literary survey on the problems
of uraemic pulmonary oedema (fluid-retention lung, etc.)

by

BENGT LINDQVIST

Accompanies Vol. 176

LUND 1964

ACTA MEDICA SCANDINAVICA
SUPPLEMENTUM 418

FROM THE MEDICAL CLINIC B (RENAL CLINIC),
UNIVERSITY OF LUND, LUND, SWEDEN

EXPERIMENTAL URAEMIC PULMONARY OEDEMA

including: criteria for pulmonary oedema
in anuric rabbits, the role of uraemia and overhydration,
and a literary survey on the problems
of uraemic pulmonary oedema (fluid-retention lung, etc.)

BY

BENGT LINDQVIST

LUND 1964

ACTA MEDICA SCANDINAVICA

has been published since 1919 as a continuation of Nordiskt Medicinskt Arkiv founded in 1869 by Axel Key The first volume of Acta Medica Scandinavica is therefore numbered LII (52)

The chief editors have been Axel Key 1869—1900 C. G Santesson 1901—1915 I Holmgren 1916—1957 and Birger Strandell 1958 to date.

Acta Medica Scandinavica publishes original research papers in the field of internal medicine. Priority will be given to authors from countries which *are represented on the editorial board. Contributors from those countries* should submit their papers to a representative of the country in question. Contributors from other countries should send their papers to the Editor at the address below

Submission of a manuscript for publication will be held to imply that the *paper has not been published elsewhere and that if accepted it will not be* republished in any other journal in the same or similar form without the Editor s written consent.

Papers will be published in English French or German at the authors choice, followed by a summary in English.

The *manuscripts shall be in final form as submitted.* They shall be typewritten with double spacing and broad left hand margin.

If a paper exceeds 16 printed pages, the cost for the excess pages shall be borne by the author No paper shall exceed 24 printed pages.

Subscription

The annual subscription to the journal, covering two volumes, each of 6 numbers is 140 Sw crowns or U S $ 27.25 *including postage* in the Scandinavian countries and in *Holland 120 Sw crowns.*

Address for subscriptions and all communications
ACTA MEDICA SCANDINAVICA
P O Box 2052 Stockholm 2

— —

Requests for duplicate copies of numbers that have gone astray can only be considered if made within a fortnight of the receipt of the following number

ACTA MEDICA SCANDINAVICA

SUPPLEMENTUM 418

FROM THE MEDICAL CLINIC B (RENAL CLINIC),
UNIVERSITY OF LUND, LUND, SWEDEN

EXPERIMENTAL URAEMIC PULMONARY OEDEMA

including criteria for pulmonary oedema
in anuric rabbits, the role of uraemia and overhydration,
and a literary survey on the problems
of uraemic pulmonary oedema (fluid-retention lung etc.)

BY

BENGT LINDQVIST

LUND 1964

ACTA MEDICA SCANDINAVICA

has been published since 1910 as a continuation of Nordiskt Medicinskt Arkiv founded in 1869 by Axel Key The first volume of Acta Medica Scandinavica is therefore numbered LII (52)

The chief editors have been Axel Key 1869—1900 C. G Santesson 1901—1915 I Holmgren 1916—1957 and Birger Strandell 1958 to date.

Acta Medica Scandinavica publishes original research papers in the field of internal medicine. Priority will be given to authors from countries which are represented on the editorial board. Contributors from those countries should submit their papers to a representative of the country in question Contributors from other countries should send their papers to the Editor at the address below

Submission of a manuscript for publication will be held to imply that the paper has not been published elsewhere and that if accepted it will not be republished in any other journal in the same or similar form without the Editor s written consent.

Papers will be published in English French or German at the authors choice, followed by a summary in English

The manuscripts shall be in final form as submitted. They shall be typewritten with double spacing and broad left hand margin.

If a paper exceeds 16 printed pages, the cost for the excess pages shall be borne by the author No paper shall exceed 24 printed pages.

Subscription

The annual subscription to the journal covering *two volumes, each of 6 numbers*, is 140 Sw crowns or U.S $ 27.25 *including postage* in the Scandinavian countries and in Holland 120 Sw crowns.

Address for subscriptions and all communications
ACTA MEDICA SCANDINAVICA
P O Box 2052 Stockholm 2

—

Requests for duplicate copies of numbers that have gone astray can only be considered if made within a fortnight of the receipt of the following number

Contents

Translated by Mrs Greta Sargeant

Printed in Sweden

LUND 1964

BERLINGSKA BOKTRYCKERIET

Introduction

In a medical renal clinic, which admits many patients with severe renal failure, pulmonary changes of the type here designated as uraemic pulmonary oedema are of great diagnostic and therapeutic importance. In close co-operation with the department of diagnostic radiography at the University clinics extensive studies has been carried out at this clinic in the last 20 years. Uraemic pulmonary oedema is a common complication of severe renal disease. Alwall & Kjellstrand, in a survey of 903 patients with an acute or chronic renal failure, found radiographic pulmonary changes of this type in 42.7 % of the cases.

The diagnosis of uraemic pulmonary oedema is based mainly on the X ray findings. Clinically the symptoms may at first be insignificant, even when the radiographic changes are considerable. Alwall and Kjellstrand point out that the problem of differentiation can make a correct in vivo-diagnosis difficult. They stress that the diagnosis is a radiographic-clinical one. The radiographic and the clinical pictures of uraemic pulmonary oedema can be complicated by such factors as ab- l raised diaphragm in intestinal paralysis, accumulation of secretion in the respiratory tract, atelectasis, bronchitis and bronchopneumonia hyperaemia, and pleural effusion, etc. Moreover many patients with uraemic pulmonary oedema in addition to their uraemia and pulmonary changes are severely ill from other causes (for instance traumatic injuries, infections and shock). For this reason they are more or less unable to co-operate at clinical examinations and the X ray examinations must be made at the bedside. At autopsy final and postmortal changes may make it difficult to draw definite conclusions as regards the changes that were present in their lungs in vivo. The variety of names used in the literature for these pulmonary changes (uraemic pulmonary oedema, fluid retention lung, uraemic pneumonitis, congestive heart failure, etc.) reflect the different concept of the pathogenesis.

The problems connected with the differential diagnosis and the patients inability to co-operate are the main factors that hamper studies of uraemic pulmonary oedema in vivo. I have therefore tried to throw some light on

A short historical survey on pulmonary oedema with special reference to renal diseases

Pulmonary edema. An effusion of serous fluid into the air vesicles and interstitial tissue of the lungs. (Dorland 1957.)

In about 1500 B.C. oedema and dyspnoea were for the first time mentioned in writing on papyrus from ancient Egypt (95). The first clinical description on pulmonary oedema in our times was given by Maloët in 1752. Surveys on pulmonary oedema were published by several authors (3, 43, 48, 121, 157, 168, 173, 217, 246, and others).

Pulmonary oedema has been described in cardiac, pulmonary, cerebral, and renal diseases, in hypertension, infections, burns, intoxications, liver cirrhosis, and other conditions.

Pulmonary oedema in renal disease was first described by Legendre (1864) and Frerichs (1851) who observed pulmonary oedema in patients with nephritis. Goodhart (1879) reported pulmonary oedema in 5 children with acute renal insufficiency. He recommended large doses of laxatives to control oedema, as digitalis usually had no great effect. Teissier (1900) and Merklen (1900) found that pulmonary oedema is common in nephrosclerosis and chronic glomerulonephritis but uncommon in subacute glomerulonephritis with nephrosis. Teissier (1900) and Whitehall (1930) observed an increased tendency to pulmonary oedema in increasing renal insufficiency. Cameron (1948) noted pulmonary oedema in 74 % at autopsy of 60 cases with chronic nephritis. Alwall & Herner (1949) showed that pulmonary oedema together with uraemia are the greatest risks to a patient suffering from acute renal failure, and that the risks of oedema in acute nephritis with oliguria are of great practical importance.

Bluemle et al. (1959), on the basis of clinical symptoms, reported an incidence of congestive failure in 100 cases of acute tubular necrosis of 15—32 %. Alwall (1960) in an account of the results of systematic X ray investigations, reported changes of the fluid retention lung type in 260 out of 607 patients (43 %) with renal insufficiency, 66 % had acute tubular necrosis. Nieth (1961) noted cardiac insufficiency and pulmonary oedema clinically or radiologically in 9 % of his cases with acute or subacute glomerulonephritis.

Langendorf & Pick (1938) published a survey of cardiac failure in acute nephritis. Ellis (1930) noted cardiac insufficiency in 20 % and Whitehall (1930) found it in 70 % of such cases. The symptoms were cardiac enlarge-

the diagnostic and pathogenetic aspects of this clinically important question by experiments on animals

A survey of the numerous reports of experimental studies in animals shows, however that even the definition of pulmonary oedema and the criteria for the diagnosis are subject to great uncertainty In view of the different opinions as regards the concept of uraemic pulmonary oedema, clinically as well as experimentally these experiments were designed to study particularly the diagnostic value of the criteria in pulmonary developed under varying experimental conditions The question of uraemia and the significance of overhydration in uraemic pulmonary oedema has also been studied by experiments in animals

CHAPTER 1

A short historical survey on pulmonary oedema with special reference to renal diseases

Pulmonary edema: An effusion of serous fluid int the air vesicles and interstitial tissue of the lungs. (Dorland 1947)

In about 1600 B.C. oedema and dyspnoea were for the first time mentioned in writing on papyrus from ancient Egypt (95). The first clinical description on pulmonary oedema in our times was given by Maloët in 1752. Surveys on pulmonary oedema were published by several authors (3, 43 48, 121 157 168, 178 217 246 and others)

Pulmonary oedema has been described in cardiac pulmonary cerebral, and renal diseases, in hypertension, infections, burns, intoxications, liver cirrhosis, and other conditions.

Pulmonary oedema in renal disease was first described by Legendre (1864) and Frerichs (1851) who observed pulmonary oedema in patients with nephritis. Goodhart (1879) reported pulmonary oedema in 5 children with acute renal insufficiency He recommended large doses of laxatives to control oedema, as digitalis usually had no great effect Teissier (1900) and Merklen (1900) found th t pulmonary oedema is common in nephrosclerosis nd chronic glomerulonephritis but uncommon in subacut glomerulonephritis with nephrosis. Tei sier (1900) nd Whitehall (1930) observed an increased tendency to pulmonary oedema in increasing renal insufficiency Cameron (1948) noted pulmonary oedema in 74 % at autopsy of 50 cases with chronic nephritis. Alwall & Herner (1949) showed that pulmonary oedema together with uraemia are the greatest risks to a patient suffering from acute renal failure, and that the risks of oedema in acute nephritis with oliguria are of great practical importance.

Bluemle et al (1959) on the basis of clinical symptoms reported an incidence of congestive failure in 100 cases of acute tubular necrosis of 15—32 % Alwall (1960) in an account of the results of systematic X ray investigations, reported changes of the fluid retention lung type in 260 *out of* 607 patients (43 %) with renal insufficiency 60 % had acute tubular necrosis Nieth (1961) noted cardiac insufficiency and pulmonary oedema clinically or radiologically in 9 % of his cases with acute or subacute glomerulonephritis.

Langendorf & Pick (1938) published a survey of cardiac failure in acute nephritis. Ellis (1936) noted cardiac insufficiency in 20 % and Whitehall (1939) found it in 70 % of such cases The symptoms were cardiac enlarge

ment hypertension dyspnoea and raised venous pressure

X ray in the diagnosis of pulmonary oedema in renal disease was first used by Ehrich & McIntosh (1932) but Zdansky (1933) and Roubier & Plachu (1934) were among the first to describe the more characteristic features Later numerous authors have reported results and discussed the X ray findings and their pathogenesis (6 10 11 16 20 21 26 31 32 38 39 43 54 60 63 64 65 91 103 105 118 120 122 123 125 126 131 132 136, 140 144 145 157 160 166 171 193 194 197 198 205 215 216 222 227 239 251 259 261 262)

The radiographic changes characteristic of pulmonary oedema in patients with renal disease have been called by many names, because of the varying views held on their pathogenesis. Some authors have used unspecific designations, such as "pulmonary oedema" (122 125) "acute pulmonary oedema" (261) "chronic pulmonary oedema" (194 251) Among the most usual designations are "pulmonary oedema in renal insufficiency "pulmonary oedema in uraemia "uraemic oedema of the lungs and uraemic pulmonary oedema (38 60 63 105 145 222) Some talk about pulmonary oedema of renal origin "nephrogenic pulmonary oedema (131 144 160) others about "uraemic lung "uraemic pneumonitis "uraemic pneumonia" pulmonary changes in uraemia "pulmonary manifestations of azotaemia (26 118 120 123 126 140 259 262) and of myocardial failure in nephritis" heart failure in acute nephritis "heart failure and pulmonary oedema" pulmonary congestion in uraemia" (54 60 196 253) Other designations are "pulmonary changes in hypertension" "pulmonary changes in nephritis (124 205)

Eventually the terms fluid lung (13 16 17) fluid retention lung (21) and "uraemic fluid lung (244) were introduced

In this survey the neutral designation uraemic pulmonary oedema will be used

Literary survey on the problems of uraemic pulmonary oedema

The *clinical picture* of clinically manifest severe uraemic pulmonary oedema resembles that of pulmonary oedema of other origin (59 91 and others) The auscultatory findings, on the other hand, may be minimal even in cases of relatively extensive radiographic changes (13, 32, 63) Pleural effusion is present in small or moderate amounts in about 50 % of the cases (59) Facial oedema is common, especially in cases of acute renal insufficiency whereas oedema of the legs is rare (20) The patients exhibit no or only slight dyspnoea and no cyanosis tachypnoea occurs but its correlation with the degree of pulmonary oedema is relatively poor In patients with uraemic pulmonary oedema, alveolar ventilation is reduced and diffusely distributed among all parts of the lung, and vital capacity declines (92) Fever is not a clinical feature (32) Tachycardia is common (11 125). Moderate anaemia and a slight decrease of serum proteins are usual features. In most cases the patients have hypertension of varying degrees, but pulmonary changes can also occur in patients who have a low blood pressure or are in shock (3, 11 18, 20 21 190) the hypertension is usually reduced or disappears after the pulmonary oedema has been brought under control by dehydration. Occasionally however the blood pressure will rise in response to removal of the oedema (3) Many overhydrated patients, with or without oedema of the lungs, show signs of cerebral oedema, such as restlessness personality changes, mental confusion, and convulsions (20 21) Ascites does not seem to be a common feature.

Uraemic pulmonary oedema in patients can be complicated by bronchitis and stagnation of secretion, atelectasis, bronchopneumonia, haemorrhages, etc., and, notably in nephrosclerosis and chronic glomerulonephritis, also by congestion as a result of cardiac insufficiency (11 18, 20 21 81 92, 102 105 163 213)

The *microscopical picture* of uraemic pulmonary oedema at autopsy has been described by several authors (31 32, 63, 126 and others) who observed hyperaemia in the lung capillaries, thickening of the alveolar septa, fibrinous or albuminous alveolitis with partial hyalinization of the intra alveolar exudate, and hyaline membranes clothing the alveolar walls. Small haemorrhages intra alveolarly and intersti

ment, hypertension, dyspnoea and raised venous pressure.

X ray in the diagnosis of pulmonary oedema in renal disease was first used by Ehrich & McIntosh (1932) but Zdansky (1933) and Roubier & Plachu (1934) were among the first to describe the more characteristic features. Later numerous authors have report ed results and discussed the X ray findings and their pathogenesis (6 10 11 16 20 21 26 31 32 38 39 43 54 60 63 64 65 91 103 105 118 120 122 123 125 126 131 132 136 140 144 145 157 160 166 171 193 194 197 198 205 215 216 222 227 239 251 259 261 262)

The radiographic changes charac teristic of pulmonary oedema in pa tients with renal disease have been cal led by many names, because of the varying views held on their pathoge nesis. Some authors have used un specific designations, such as "pulmonary oedema (122 125) "acute pul monary oedema" (261) chronic pul monary oedema (194 251) Among the most usual designations are "pul monary oedema in renal insufficien cy" pulmonary oedema in uraemia" uraemic oedema of the lungs" and "uraemic pulmonary oedema (38 60 63 105 145 222) Some talk about pulmonary oedema of renal origin" nephrogenic pulmonary oedema" (131 144 160) others about "uraemic lung "uraemic pneumonitis" "uraemic pneumonia" "pulmonary changes in uraemia" pulmonary manifesta tions of azotaemia (26 118 120 123 126 140 259 262) and of myocardial failure in nephritis" "heart failure in acute nephritis" "heart failure and pulmonary oedema" pulmonary con gestion in uraemia (54 60 196 253) Other designations are "pulmonary changes in hypertension "pulmonary changes in nephritis (124 205)

Eventually the terms "fluid lung" (13 16 17) "fluid retention lung" (21) and "uraemic fluid lung" (244) were introduced

In this survey the neutral designa tion "uraemic pulmonary oedema will be used.

Literary survey on the problems of uraemic pulmonary oedema

The *clinical picture* of clinically manifest severe uraemic pulmonary oedema resembles that of pulmonary oedema of other origin (59 91 and others) The auscultatory findings, on the other hand, may be minimal even in cases of relatively extensive radiographic changes (13, 32, 63) Pleural effusion is present in small or moderate amounts in about 50 % of the cases (59) Facial oedema is common, especially in cases of acute renal insufficiency whereas oedema of the legs is rare (20) The patients exhibit no or only slight dyspnoea and no cyanosis tachypnoea occurs but its correlation with the degree of pulmonary oedema is relatively poor In patients with uraemic pulmonary oedema, alveolar ventilation is reduced and diffusely distributed among all parts of the lung, and vital capacity declines (92) Fever is not a clinical feature (82) *Tachycardia* is common (11 125) Moderate anaemia and a slight decrease of serum-proteins are usual features. In most cases the patients have hypertension of varying degrees, but pulmonary changes can also occur in patients who have a low blood pressure or are in shock (3, 11 18, 20, 21 196) the hypertension is usually reduced or disappears after the pulmonary oedema has been brought under control by dehydration. Occasionally however the blood pressure will rise in response to removal of the oedema (3) Many overhydrated patients, with or without oedema of the lungs, show signs of cerebral oedema, such as restlessness, personality changes, mental confusion, and convulsions (20 21) Ascites does not seem to be a common feature.

Uraemic pulmonary oedema in patients can be complicated by bronchitis and stagnation of secretion, atelectasis, bronchopneumonia haemorrhages, etc., and, notably in nephrosclerosis and chronic glomerulonephritis, also by congestion as a result of cardiac insufficiency (11 18 20 21 81 92, 102, 105, 163 213)

The *microscopical picture* of uraemic pulmonary oedema at autopsy has been described by several authors (31 32, 63 126 and others), who observed *hyperaemia in the lung capillaries*, thickening of the alveolar septa, fibrinous or albuminous *alveolitis* with partial hyalinization of the intra-alveolar exudate, and hyaline membranes clothing the alveolar walls. Small haemorrhages intra-alveolarly and interstit

ment, hypertension dyspnoea and raised venous pressure.

X ray in the diagnosis of pulmonary oedema in renal disease was first used by *Ehrich & McIntosh (1932)* but Zdansky (1933) and Roubier & Plachu (1934) were among the first to describe the more characteristic features. Later numerous authors have reported results and discussed the X ray findings and their pathogenesis (6 10 11 16 20 21 26 31 32 38, 39 43 54 60 63 64 65 91 *103* 105 *118*, *120* *122* *123* *125* *126* 131 *132* 136 *140* 144 145 157 160 166 171 193 194 197 198 205 215 216 222 227 230 251 259 261 262)

The radiographic changes characteristic of pulmonary oedema in patients with renal disease have been called by many names, because of the varying views held on their pathogenesis. Some authors have used unspecific designations such as pulmonary oedema (122 125) acute pulmonary oedema (261) "chronic pulmonary oedema (194 251) Among the most usual designations are "pulmonary oedema in renal insufficiency "pulmonary oedema in uraemia "uraemic oedema of the lungs and uraemic pulmonary oedema (38 60 *63 105 145 222)* Some talk about pulmonary oedema of renal origin" nephrogenic pulmonary oedema" (131 *144* 160) others about "uraemic lung "uraemic pneumonitis uraemic pneumonia" *pulmonary changes* in uraemia "pulmonary manifestations of azotaemia" (26 118 120 123 *126 140 259 262)* and of myocardial *failure in nephritis"* "*heart failure* in acute nephritis" "heart failure and pulmonary oedema" pulmonary congestion in uraemia (54 60 196 253) Other designations are "pulmonary changes in hypertension pulmonary changes in nephritis (124 205)

Eventually the terms fluid lung" (13 16 17) fluid retention lung" (21) and uraemic fluid lung (244) were introduced.

In this survey the neutral designation *uraemic* pulmonary oedema" will be used.

nary oedema of hilar type occurs only in association with some degree of uraemia. Borgström et al. (1960) also postulated that the hilar type signifies renal disease. Several authors have however described cases of cardiac insufficiency with pulmonary changes of hilar type (60, 64, 10, 132, 104). Gould & Torrance (1955) found that the hilar type was most frequent in patients with renal diseases but that the borderlines between the said types of pulmonary changes were not distinct.

The radiographic picture is often complicated, since in patients with renal disease several pathological processes occur at the same time, such as renal and cardiac insufficiency, bronchopneumonia, haemorrhages and atelectasis. The heart is often enlarged and the diaphragm raised because of abdominal distension. Under such conditions the diagnosis and the grading of pulmonary oedema are difficult (20, 21, 30, 122, 108). In rare cases, X-ray can be normal in acute pulmonary oedema with protein-rich transudate even in patients who die from suffocation due to profuse frothing (39).

Pathogenesis. According to Altschule (1954) the fundamental disorder in pulmonary oedema seems to be a rate of transudation from the pulmonary capillaries that exceeds the reabsorptive capacity of the pulmonary lymphatics. The following factors contribute to this imbalance: 1) Increased transudation caused by a) elevated capillary pressure in the lungs, b) increased filtering area in the lungs, c) large blood flow in the lungs, d) lowered plasma protein level, e) increased capillary permeability and f) bronchospasm. 2) Decreased reabsorption owing to impaired lymphatic function. 3) Increased total extracellular fluid volume. According to Macheen (1960) no single mechanism is responsible for all forms of pulmonary oedema.

Uraemic pulmonary oedema occurs in a variety of renal diseases, such as acute and chronic glomerulonephritis, tubular necrosis, nephrosclerosis, and polycystic kidneys. The incidence in the various renal diseases has not been made clear. Alwall (1960 and 1963) found that the incidence of "fluid lung" was about equally high in anuric-oliguric patients with tubular necrosis (39 % of 398 patients), glomerulonephritis (40 % of 115 patients), pyelonephritis (46 % of 24 patients) and postrenal obstruction (30 % of 52 patients) but slightly higher in patients with nephrosclerosis (70 % of 10 patients). The incidence was influenced by the duration and degree of severity of the disease (milder cases are less exposed to the risk of overhydration), the presence of overhydration at admission (when most cases of "fluid lung" occurred), a tendency to shock (more fluid had to be given in such cases) and the presence of pneumonia and other conditions which rendered a correct X-ray diagnosis difficult. The question whether the pathogenesis of pulmonary oedema differs in different renal diseases has not been settled.

Some of the factors that are considered to play a part in causing uraemic pulmonary oedema are

tially (59 118) bronchiolitis (75 255) and focal atelectasis with emphysema (126) *have also been observed* Some have noted a profuse amount of protein in the alveolar exudate (e.g 126) *but a low concentration of protein has* also been reported (118)

The microscopical changes are not *pathognomonic.* Similar changes are seen in radiation pneumonia virus pneumonia rheumatic pneumonia respiration atelectasis *in the* newborn lupus erythematosus periarteritis nodosa and after inhalation of toxic substances *(e.g 26 31 32 106)* Bronchiolitis can occur in association with infection or inhalation of chemical substances *(75) Bass* et al assumed that in the above-mentioned conditions there would be disturbances in permeability

The *radiographic abnormalities* described as *typical of uraemic pulmonary* oedema are bilateral central diffuse changes, most marked in the hilar region *surrounded by free peripheral* zones of normal parenchyma (222 261) Unilateral changes may occur (103 125 194 and others) which may be caused by bed rest in the side position (21) The changes can alter their *appearance or disappear within a* short time (6 16 17 18 103 171 197 251) Pleural effusion is invariably *present but usually found in* small amounts at examination in the side-position (16 20 38) There is no correlation *between* the amount of fluid in the pleura and the degree of pulmonary oedema assessed radiographically *(39)* Oedema of the lungs can be found both in patients *with normal sized* hearts and in patients with enlarged hearts (261) Out of 200 patients (also described in *Alwall s et al series) with* radiographic evidence of uraemic pulmonary oedema half had enlarged hearts as a result *of dilatation hyper*trophy or pericarditis (30) In cases of massive pulmonary oedema the changes extend *peripherally and the* free zones disappear after dehydration the changes disappear first at the periphery (20 21)

The "batswing shadow" (125) seen *in the* radiograph which has often been described as characteristic, has been given several explanations Insufficient flow of lymph in the hilar region (71) stagnation of blood in the hilar region (61) reduced blood flow peripherally *(212)* the bronchial vessels do not extend peripherally in the lung (38) the function of the central *parts of the lung is different from that* of the cortex (122) or that the whole lung undergoes a change but that the thick *layers of the central* parts are more clearly visible in the radiograph (20 21)

The question whether it is possible to distinguish radiographically a lung oedema of cardiac origin from a lung *oedema of renal origin* seems to be left open. Both Zdansky (1933) and Goodrich (1948) considered that pulmonary oedema with hilar extension in the radiograph would be acute pulmonary oedema and with *extension* basally and peripherally it would be chronic pulmonary oedema notably *cardiac insufficiency* Rendich et al. (1941) considered that typical pulmo-

extravascular fluid volume was increased in uraemic patients without clinically demonstrated oedema. Altschule (1954) stated that the increase in extracellular fluid which often occurs in, for instance, acute nephritis results in a tendency to pulmonary oedema; the explanation would be that, except for the skin, the lung is the organ that is richest in collagen tissue, in which extracellular fluid accumulates.

Cardiac failure: Reports found in the older literature concern mainly patients in the terminal stage of chronic renal disease, who had marked hypertension, hypertrophy of the heart, and severe electrocardiographic changes. This led many authors to attach special significance to cardiac insufficiency as the cause of pulmonary oedema in renal disease (e.g. 32, 61, 132, 146, 180, 215, 221). Anthonisen considered that many facts favour the theory that pulmonary oedema in chronic renal disease is a result of cardiac failure, in that arteriosclerotic or hypertensive heart-disease, uraemic pericarditis, electrolyte disturbances, and, perhaps, toxic substances in uraemia, as well as anaemia have an inhibitory action on the heart; while, at the same time, the demands on the heart are increased by fever, anoxia, muscular tension, twitchings, and anaemia. De Bass et al. (1956) believed that the main cause would be electrolyte-fluid retention resulting from renal insufficiency, hypervolaemia, and secondary heart failure.

Other authors claim, however, that pulmonary oedema occurs in patients with a normal-sized heart, a normal electrocardiogram, normal clinical findings, and a normal heart at autopsy; digitalis has no effect (3, 6, 10, 11, 26, 54, 60, 61, 142, and others). Various types of radiographic lung-changes occur in primary cardiac insufficiency and in renal insufficiency (38, 103, 105). As regards the fact that an increased heart size in pulmonary oedema returns to normal when the oedema is brought under control (241), Altschule (1954) made the following comment: The enlargement of the heart need not signify insufficiency but can be secondary to fluid retention.

Cardiac catheterizations have been reported in only a few cases with pulmonary oedema, predominantly in patients with acute glomerulonephritis. Davies (1951) noted a normal cardiac output. Eichna et al. (1951) observed that the cardiac output was normal but the pressure in the pulmonary artery and right atrium was raised. Morita (1957) reported raised cardiac output, raised pressure in the pulmonary artery, the right atrium, and the lung capillaries, and a reduced arteriovenous oxygen difference. De Fazio (1959) noted a markedly increased cardiac output, raised pressure in the pulmonary artery and the lung capillaries, but only slightly raised pressure in the right atrium. Some of our overhydrated uraemic patients with "fluid retention lung" and arterial hypertension showed virtually normal pressure (catheterizations performed by Inge

Uraemia Oedema of the lung in association with renal disease is predominantly reported in patients with uraemia. In rare cases, however pulmonary oedema *of this type occurs in* patients with slight uraemia (21 216) and in patients with suspected renal damage without uraemia. Cases of the latter type have been described in patients with malignant hypertension (65 115 124 125 132) and acute glomerulonephritis (103 255) during pregnancy (132) *and in newly operated* patients (136)

Toxic substances of various kinds are considered to cause an increase in capillary permeability in uraemia (13 38 63 103 105 118 122 262 and others) Uraemic pulmonary oedema can disappear if the uraemia is reduced by dialysis, even if the body weight increases (120 182)

There is no correlation between the degree of uraemia and the extent of the pulmonary changes (21 63 216) *At X ray examination of a large number* of patients with severe uraemia only a few were found to have changes of pulmonary-oedema type (216) Oedema of the lungs in uraemic patients occurs *among those who fall ill* with acute anuria as well as in those who have had uraemia for many years (20 21) If a uraemic patient with pulmonary oedema is dehydrated the pulmonary changes disappear even though the uraemia continues to increase (13 18 20 21) Nor has any *correlation* been demonstrated between the concentration of urea, creatinine, uric acid acidosis etc. *and the presence* of pulmonary oedema.

Fluid retention Exogenous overhydration resulting from, for instance parenteral infusions or *endogenous* overhydration due to inadequate elimination of the water produced in catabolism can cause pulmonary oedema in renal insufficiency (6 10 13, 16, 17 18 19 20 21 103 194 216 *and* others) Dangerous fluid therapy without weight-control in cases of acute nephritis (to make diuresis recommence or increase) can lead to fatal oedema (10) *The radiographic* changes consistent with pulmonary oedema can disappear in response to dehydration (6 10 11 13 16 17 20 21 160 261) The pulmonary changes can be made to *disappear in a few* hours by diarrhoea induced with sodium sulphate or sorbitol (6, 10 11 13 16 18, 20 21) or by ultrafiltration of the blood in vivo (6 10 11 12 13 16 17 19 20 21)

Alwall (1952) and Alwall Lunderquist & Olsson (1953) referring to the *above mentioned* clinical results (Alwall & Olsson) and experimental results in animals (Alwall & Lunderquist) proposed that the terms uraemic lung etc. should be replaced by fluid *lung as this designation* would give information about the pathogenesis and point to the adequate treatment. Alwall later (1963) used the term fluid retention lung" Few studies on the *body s fluid spaces* in pulmonary oedema have been reported. A markedly increased extravascular *alcohol* space in the lung of a patient with pulmonary oedema has been observed (122)

Nickel et al. (1953) noted that the

extravascular fluid volume was increased in uraemic patients without clinically demonstrated oedema. Altschule (1954) stated that the increase in extracellular fluid which often occurs in, for instance, acute nephritis results in a tendency to pulmonary oedema the explanation would be that, except for the skin, the lung is the organ that is richest in collagen tissue, in which extracellular fluid accumulates.

Cardiac failure Reports found in the older literature concern mainly patients in the terminal stage of chronic renal disease, who had marked hypertension, hypertrophy of the heart, and severe electrocardiographic changes. This led many authors to attach special significance to cardiac insufficiency as the cause of pulmonary oedema in renal disease (e.g. 32 64 132 145 160 216 221) Anthonisen considered that many facts favour the theory that pulmonary oedema in chronic renal disease is a result of cardiac failure, in that arteriosclerotic or hypertensive heart-disease, uraemic pericarditis, electrolyte disturbances, and, perhaps, toxic substances in uraemia, as well as anaemia ha e an inhibitory action on the heart, while, at the same time, the demands on the heart are increased by fever anoxia, muscular tension, twitchings, and anaemia. De Bass et al. (1956) believed that the main cause would be electrolyte-fluid retention resulting from renal insufficiency hypervolaemia, and secondary heart failure.

Other authors claim however that pulmonary oedema occurs in patients with a normal-sized heart, a normal electrocardiogram normal clinical findings, and a normal heart at autopsy digitalis has no effect (3 6, 10 11 26 54 60 91 142 and others) Various types of radiographic lung-changes occur in primary cardiac insufficiency and in renal insufficiency (58, 103, 105) As regards the fact that an increased heart size in pulmonary oedema returns to normal when the oedema is brought under control (?41) Altschule (1954) made the following comment The enlargement of the heart need not signify insufficiency but can be secondary to fluid retention.

Cardiac catheterizations have been reported in only a few cases with pulmonary oedema, predominantly in patients with acute glomerulonephritis. Davies (1951) noted a normal cardiac output. Eichna et al. (1954) observed that the cardiac output was *normal* but the pressure in the pulmonary artery and right atrium was raised. Morita (1957) reported raised cardiac output, raised pressure in the pulmonary artery the right atrium and the lung capillaries and a reduced arteriovenous oxygen difference. De Fazio (1950) noted a markedly increased cardiac *output*, raised pressure *in the* pulmonary artery and the lung capillaries, but only slightly raised pressure in the *right* atrium. Some of our overhydrated uraemic patients with fluid retention lung" and arterial hypertension showed *virtually* normal pressure (catheterizations performed by Inge

Edler M D) in the right atrium as well as in the pulmonary artery and capillaries (Alwall 1963) Anthonisen (1960) found a normal cardiac output in uraemia without oedema

Kassirer & Schwartz (1961) stressed the difference between pulmonary oedema resulting from primary heart failure and pulmonary oedema occur ring in salt and water rentention in acute glomerulonephritis, acute renal failure, anaemia, steroid overdosage, and beri beri In contrast to heart fail ure, the last mentioned conditions are attended with normal or increased car diac output small arterio-venous oxy gen differences and normal or reduced total red cell volume and digitalis has no beneficial effect on pulmonary oedema of the latter cause. The authors therefore considered it doubt ful whether one can speak about heart failure in these conditions.

Hypertension This is a common phe nomenon in uraemic pulmonary oede ma (3 20 23 196 and others) The aetiological role of hypertension in pulmonary oedema remains to be clar ified, but whatever mechanisms are ul timately found it is clinically evident that hypertension is important in the genesis of pulmonary oedema (3) Lichna et al (1954) found a low pres sure in the heart, normal blood vol ume, and a seemingly normal heart in a patient with pulmonary oedema They considered that these findings could be attributable to a redistribu tion of the blood volume to the lungs from other vascular areas. A similar assumption was made in connection with pulmonary oedema of renal type in a hypertensive patient with phaeo-chromocytoma (105) In animals, pul monary oedema with a similar mechan ism can be induced by injection of adrenaline. The following arguments have been presented against hyperten sion as the cause of circulatory con gestion in acute glomerulonephritis 1) The blood pressure seldom rises above 180/100 mm Hg in these cases 2) There is no correlation between hyper tension and circulatory congestion 3) Some patients have circulatory con gestion without hypertension (142 196)

Some other pathogenic factors Hyp-oxia has been thought to play a part in causing increased permeability in the lung capillaries and pulmonary oede ma in renal disease (32 105) Marked stagnation of secretion, atelectasis and bronchopneumonia are common in uraemic patients (60 85 92) with dimi nished pO as a result (81) Compli cating infection in the lungs and tachy pnoea have been considered as causal factors in pulmonary oedema asso-ciated with renal disease (60)

Hypoxia does not seem to be the whole explanation of pulmonary oede-ma since a vicious circle would rapid ly be set up and thus, every pulmona ry oedema would always become ir reversible (103) Normal oxygen satu ration occurs even in uraemic patients with slight pulmonary oedema (56) and many patients with uraemia have normal pO_2 (21) When uraemic pa tients have hypoxia it is in many cases

due to causes other than pulmonary oedema (81)

Anaemia has been mentioned as a factor concerned in the causation of uraemic pulmonary oedema (64) Patients with chronic anaemia of various origin have a high cardiac output and are likely to develop pulmonary oedema after infusions (233) The mechanism has not been made clear

Cerebral damage has been suggested as a causal agent in uraemic pulmonary oedema (103 105 122) The cerebral factor would act via a change in the vagal tone (51) or respiration (3) However cerebral oedema occurs independently of pulmonary oedema (20)

Changes in the amount of plasma protein are relatively slight in most renal patients with pulmonary oedema. The lack of correlation between pulmonary oedema and hypoproteinaemia is evident from the fact that pulmonary oedema seldom occurs in nephrosis (181 238)

Literary survey of experimental uraemic pulmonary oedema

Experimental pulmonary oedema was first induced by Cohnheim & Lichtheim in 1850—1860 Several surveys of the literature on experimentally produced pulmonary oedema have been published (e g 108 202 246)

Vischer et al. (1956) summarized the various experimental methods of producing pulmonary oedema as follows 1) Primary haemodynamic alterations (operations on the heart and the large vessels, hypervolaemia arterial hypotension, embolism) 2) Alterations of the central nervous system (brain lesions compression or stimulation) 3) Alterations of the peripheral nervous system (vagotomy faradic stimulation of lung root) 4) Alterations of the respiratory system (air way obstruction, hypoxia, drowning intratracheal fluids etc.) 5) Pharmacological effects (muscarine, histamine adrenaline, lung irritant gases, bile salts, barbiturates, and others) 6) Miscellaneous (hyperthermia sensitivity phenomena mechanical irritation of the bronchus)

The tendency to develop pulmonary oedema varies from one animal species to another Welch as early as 1878 observed that rabbits were more apt to develop lung oedema than were dogs The pathogenesis of experimentally produced pulmonary oedema is not always the same the history of the methods used in this work will be surveyed at the beginning of each chapter

Alwall (1952) Alwall & Lunderquist (1953) Lunderquist (1953) and Alwall (1954 196?) in experiments with rabbits whose ureters had been ligated tried to induce slowly developing pulmonary oedema which could give a radiographic picture similar to the clinical picture of uraemic pulmonary oedema Overhydration was not sufficient. The toxic effect of Narkotal (Alwall et al. 1949) was therefore utilized as a basis Short anaesthesia induced with Narkotal and overhydration gave a high frequency of radiographic pulmonary changes (Narkotal uraemia fluid lung) within a few days in anuric rabbits. A detailed account of these experimental series will be found in Chapter 10

Borgström Ising Linder & Lunderquist (1960) aimed at obtaining a radiographic picture which they regarded as typical of uraemic pulmonary oedema The uremic pulmonary edema is characterized by central changes, surrounded by a free peripheral zone of normal lung parenchyma 2 to 4 cm in thickness and slightly broader at the lobe limits. A similar radiographic picture was obtained in dogs, when gall

salts were injected into the bronchial artery at catheterisation. Injection of gall salts into the pulmonary artery or clamping of the aorta produced radiographic pulmonary oedema which, however did not show the above-stated typical picture. After injection of gall salts into the bronchial artery no enlargement of the heart, insignificant pleural effusion, insignificant oedema in the alveoli or air passages, and normal pressure in the lung capillaries were noted, but hypertension in the pulmonary artery and periarterial oedema were obtained. The pulmonary oedema induced by injection of gall salts into the bronchial artery showed such a good radiographical, microscopical, and clinical resemblance to the radiographic uraemic pulmonary oedema described in man that the authors formed the hypothesis that a change in the permeability of the bronchial capillaries may be the prime aetiological factor in the production of uraemic pulmonary oedema. Consequently a reduction in the osmotic pressure of the blood produced by overhydration and/or a complicating left heart failure would be enhancing factors.

Changes in the serum-electrolyte concentration in animals with acute anuria have been described by for instance Harrison et al. (1932)

Technique

Surveys of methods for measuring the degree of experimental pulmonary oedema have been published by Poulsen (1954) and Visscher Haddy & Stephens (1956)

According to Visscher et al 9 methods are available for evaluation of the oedema state 1) *Auscultation of the chest* 2) Observation and collection of frothy fluid in the larger air ways during life 3) X ray density studies during life 4) Gross observation at autopsy 5) Microscopical observation of autopsy material 6) Measurements of lung weight to body or other organ weight ratios at autopsy 7) Chemical analysis of extravascular extracellular fluid space in the lung at autopsy 8) Changes in electrical resistance of the lung parenchyma 9) *Weight changes in the* isolated perfused organ. Poulsen mentioned some of these methods and added 10) The proportion of the lung volume to body weight 11) Dry matter determination of the lung he also mentioned that 12) Drinker (1947) measured the amount of lymph flowing from the lung and used this as a measure of the amount of fluid penetrating through the walls of the capillaries and passing into the lymph vessels.

In the present studies which were made on rabbits, the following 10 methods were used in determining the degree of pulmonary oedema 1) Assessment of the appearance and behaviour of the animals 2) Respiratory rate 3) Electrocardiographic examination 4) X ray of the lungs 5) Gross examination of the lungs 6) Estimation of *froth in the air ways post mortem* 7) Measurements of lung weight, absolute and in relation to body weight 8) Determination of the water content of the lung 9) Microscopical examination of the lungs and 10) Lung volume, in a few experiments.

Rabbits weighing 2.20—2.49 kg, were used all were of the same breed, but with coats of different colours, and of both sexes. Each animal was placed alone in a cage, with free access to water and was given food in the form of pellets. The pellets contained corn, wheat bran, oil concentrates, fish meal, meat meal, Alfalfa leaf meal, skim milk powder bone meal and calcium and vitamins (A, B_1, D_3) On this *diet the rabbits gained satisfactorily* in weight. By daily checking of the body weight and by the clinical picture the well being of each animal was ascertained. 90 % of the rabbits were kept in their cages for at least 1 (1 —30) days before the beginning of the experiments.

The *appearance and behaviour of the animals* The power of reaction and the wakefulness of the animals could be assessed by for instance spontaneous movements, nose movements the position of the head and the reaction to different *stimuli such as touching* the nose blowing into the outer ear

and the corneal reflex. The presence of cerebral disorders could be established by unsteady gait, nystagmus, exophthalmos, restlessness, twitchings, convulsions, and loss of reflexes. Posture respiration, and the size of the orifices of the nose gave information about respiratory difficulties. Anaemia, cyanosis, and the filling of the vessels were ascertained by studying the conjunctivae and the mucous membranes of the nose and mouth. When they had been taken ill, the animals lay flat on their abdomens with paws outstretched, or remained lying in the side position without trying to rise. Stethoscopic examination was not made. This method was by Luisada & Sarnoff (170) considered to be unsatisfactory

Respiratory rate was measured before and after procedures aimed at inducing pulmonary oedema (infusions, etc.) possibly in the sitting position.

The *heart-rate and electrocardiograms* were recorded with the Mingograf (Elema Schönander Stockholm) Changes in the normal electrocardiogram of rabbits have been described by for instance, Levine (1942) and Lepeschkin (1957) Levine found that the heart-rate in rabbits t rest was 225 (174—282) beats per minute and that an inverted T wa wa present in only 1 of 23 cases in lead I in naturally sitting rabbits

Electrocardiograms were recorded before ligation of the ureters as well as before and after procedures to induce pulmonary oedema. Some ten heart beats were registered in one lead Trembling and movements of the animals and respiration could produce disturbances. It was sometimes difficult to find optimal positions for the electrodes.

Chest radiographs were taken in the anteroposterior view before ligation of the ureters, before and after procedures to induce pulmonary oedema, and before the animals were bled to death for the purpose of demonstrating pulmonary changes. A few rabbits which died spontaneously were examined immediately after death. Examinations in the lateral position for the presence of pleural fluid in vivo were made in a few series.

Chest radiographs in experimentally produced pulmonary oedema have been published by Luisada (1918) Alwall, Lunderqui t et al. (1952—1963) (also in the lateral position) and Csákány (1960) As an argument against the use of chest X-ray Visscher et al. (246) referring to Westermarck (1918) stated that distended blood vessels may be visible lung hadows, ordinarily interpreted as evidence of pulmonary oedema, and that X ray evidence of massi e lung oedema occurs only when the oedema tate has approached lethal degrees.

Our laboratory X-ray apparatus, which is f tted with an ptical centering device, allows exposures of 0.2 second at 52 kV and 570 mA. The rabbit wa fixed on table nd enclosed in iron plating, with a window cut out over the chest, nd wa thus held in the proper position. The film wa taken with the rabbit in the hanging position (Fig. 1) the tube-film distance wa 100 cm. The technique has been devised by Alwall & Lunderquist (1952) and will be described in detail by Lunderquist in 1964 (in the press)

Dr Anders Lunderquist at the X ray Department I Lund (Head Olle Ols-

CHAPTER 4

Technique

Surveys of methods for measuring the degree of experimental pulmonary oedema have been published by Poulsen (1954) and Visscher Haddy & Stephens (1956)

According to Visscher et al., 9 methods are available for evaluation of the oedema state 1) Auscultation of the chest 2) Observation and collection of frothy fluid in the larger air ways during life 3) X ray density studies during life 4) Gross observation at autopsy 5) Microscopical observation of autopsy material 6) Measurements of lung weight to body or other organ weight ratios at autopsy 7) Chemical analysis of extravascular extracellular fluid space in the lung at autopsy 8) Changes in electrical resistance of the lung parenchyma 9) Weight changes in the isolated perfused organ Poulsen mentioned some of these methods and added 10) The proportion of the lung volume to body weight 11) Dry matter determination of the lung he also mentioned that 12) Drinker (1947) measured the amount of lymph flowing from the lung and used this as a measure of the amount of fluid penetrating through the walls of the capillaries and passing into the lymph vessels.

In the present studies, which were made on rabbits, the following 10 methods were used in determining the degree of pulmonary oedema 1) Assessment of the appearance and behaviour of the animals 2) Respiratory rate 3) Electrocardiographic examination 4) X ray of the lungs 5) Gross examination of the lungs 6) Estimation of froth in the air ways post mortem 7) Measurements of lung weight, absolute and in relation to body weight 8) Determination of the water content of the lung 9) Microscopical examination of the lungs and 10) Lung volume, in a few experiments.

Rabbits weighing 2.20—2 49 kg, were used all were of the same breed, but with coats of different colours, and of both sexes. Each animal was placed alone in a cage, with free access to water and was given food in the form of pellets. The pellets contained corn, wheat bran, oil concentrates, fish meal, meat meal, Alfalfa leaf meal, skim milk powder bone meal and calcium and vitamins (A B_2, D_3) On this diet the rabbits gained satisfactorily in weight. By daily checking of the body weight and by the clinical picture the well being of each animal was ascertained. 90 % of the rabbits were kept in their cages for at least 1 (1—30) days before the beginning of the experiments.

The appearance and behaviour of the animals The power of reaction and the wakefulness of the animals could be assessed by for instance spontaneous movements, nose movements the position of the head and the reaction to different stimuli such as touching the nose, blowing into the outer ear

Bloodletting was chosen in our experiments after comparison to, for instance, electrocution and intravenous injection of air or Narkotal. It does not seem advisable to use methods that could cause or aggravate pulmonary oedema, haemorrhages hyperaemia, or atelectasis. On the other hand, in my experience bloodletting can be made more or less complete which may influence the autopsy findings (the blood supply in the vessels and the weight of the lungs); the preparations are also fairly time-consuming and many of our animals died spontaneously before the planned bloodletting could be started.

The following technique was used at autopsy. When breathing had ceased the trachea was ligated tightly. The chest was opened with scissors by cutting off the ensiform process and some of the lower ribs. The heart was in most cases beating at the beginning of the autopsy. The fluid in the pericardial sac was sucked out by means of a small pipette. The entire anterior portion of the chest was then cut open and removed together with the sternum and the anterior parts of the ribs. The root of the lung was ligated bilaterally including vessels and bronchi. The pleural fluid was sucked out of the pleural cavities. The heart-lung preparation was freed caudally and lifted out of the chest. The stretched trachea was cut open and examined for the presence of frothy fluid. The trachea, the oesophagus, and other attachments were then severed. Each lung was cut off separately from the heart lung preparation and placed in an open beaker of thin aluminium foil. The thread around the root of the lung together with vessels and bronchial portions outside the lung were cut away. The lungs were examined macroscopically and weighed. The water content of the lung was estimated by examination of the cut surface through the lung and by judging how easily fluid could be expressed, and was coded as + ++ and +++. The left lung was used for determination of the water content. From the most markedly changed parts of the right lung, pieces were taken for microscopical examination; these were usually of hazelnut size, on a few occasions consisting of whole lobes. If no gross pulmonary changes were seen, and when microscopical examination was considered warranted, pieces were taken from the basal and hilar parts of the right lower lobe in which, empirically, the incidence of pulmonary changes is highest. The pieces were fixed in 10 % formalin. The amount of peritoneal fluid was measured by pouring the abdominal contents into a dish after cutting open the abdomen and, after a little while, removing the viscera so that only the peritoneal fluid remained in the dish.

Gross observation at autopsy comprised the size of the lungs, the moisture of the surfaces, and the appearance of the lung surface, the latter being the most important factor. The lung surface showed the following features in different conditions.

In normal lungs the surface was smooth and pink.

Areas of atelectasis were in most cases seen in both lower lobes, sometimes in the smaller lobes and occasionally locally at the lung borders. The lungs were small in cases of extensive atelectasis. The colour was bluish-purple or brown, occasionally purple with white spots.

Areas of pneumonia were as a rule reddish brown and clearly defined.

Hyperaemia was seen in animals which had died spontaneously or after incomplete bloodletting; the lung surface was dark red and blood ran profusely from the section surface.

Haemorrhages were manifested by small red dots, in a few cases spots, or rarely large areas of bleeding. In

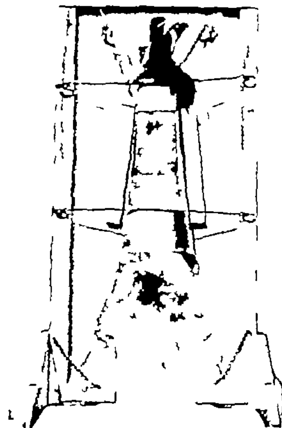


Figure 1 The position of the rabbit fixed to the table during X ray examination. The animal s back is enclosed in a metal shield provided with a window

son M D) examined the films without knowing from which series they were taken but being informed of the *amount of pleural effusion and ascites* at autopsy Radiographic changes consistent with pulmonary oedema and changes of other type were recorded. *The extent to which the changes were* conditioned by factors other than pulmonary oedema was not assessed, however The heart size was estimated and graded as "0 + ++ and +++

The following typical set of pictures *will serve as an example*

Fig 3 shows the *chest film of a* normal rabbit

Figs 4 5 6 and 7 show different degrees of pulmonary oedema coded as "+ ++ *and* +++"

Fig 8 shows pneumonia in the upper lobes of a vagotomized rabbit

Fig 9 shows atelectasis in profuse pleural effusion

Fig 10 shows large pulmonary haemorrhages

Fig 11 shows cardiac enlargement +++ without any pulmonary change.

Gross observation at autopsy

Durlacher et at. (1950) examined the lung weight after seven *different* methods of killing the rabbits (bloodletting, ether anaesthesia, intravenous injection of air electrocution rabbit punch, $MgSO$ intravenously and Nembutal intravenously) Only a slight *difference* in the weight of the lungs, irrespective of manner of death, was noted if the rabbits were examined *immediately after* death. In *rabbits* examined after lying dead for 3 hours the lung weight did not rise in those killed by bloodletting but increased markedly after all the other methods, and most markedly *after death from acute barbiturate poisoning*

Injection of 275 mg of 'Narkotal intravenously gave in our experiments hyperaemia in 4 rabbits examined 10 minutes after death, and *massive pulmonary oedema* in 4 rabbits examined about 25—30 minutes after death. Chest X ray examinations made about 5 minutes post mortem (2 animals had then frothy fluid in the nose) *showed no abnormalities.* Pulmonary oedema can possibly arise very quickly after death, but the *possibility that pulmonary* oedema can be present even if the radiograph is normal, should always be *considered.*

Bloodletting was chosen in our experiments after comparison to for instance, electrocution and intravenous injection of air or \ rkotal. It does not seem advisable to use methods that could cause or aggravate pulmonary oedema, haemorrhages, hyperaemia, or atelectasis. On the other hand, in my experience bloodletting can be made more less complete which may influence the autopsy findings (the blood supply in the essel and the weight of the lungs) the preparations are also fairly time-consuming and many of our animals died spontaneously before the planned bloodletting could be started.

The following technique was used at autopsy When breathing had ceased the trachea was ligated tightly The chest was pened with scissors by cutting off the en-af rm process and some of the lower ribs. The heart was in most cases beating at the beginning of the a topsy The fluid in the pericardial sac was sucked out by means of a small pipette. The entire anterior portion of the chest was then cut open and removed together with the sternum and the anterior parts of the ribs. The root of the lung was ligated bilaterally including vessels and bronchi. The pleural fluid wa sucked out of the pleural cavities. The heart-lung preparation was freed caudally and lifted out of the chest. The tretched t chea was cut open and examined fo the presence of frothy fluid. The trachea, the oesophagus, and other ttachments wer then severed. Each lung was cut off separately from the heart lung preparation and placed in n open beaker of thin luminium foil. The thread around the root of the lung together with vessels and bronchial portions outside the lung were cut away. The lungs were examined macroscopically and weighed. The water content of the lung was estimated by examination of the cut surface through the lung and by judging how easily fluid could be expressed, nd was coded as + ++ and +++. The left lung w s used f determination f the wate content. From the most markedly changed parts of the right lung, pieces were taken for microscopical examination these were usually of hazelnut size, on a few occa sions consisting of whole lobes. If no gross pulmonary changes were seen, and when microscopical examination was considered warranted, pieces were taken from the basal and hilar parts of the right lower lobe in which, empirically the incidence of pulmonary changes is highest. The pieces were fixed in 10 % formalin. The amount f peritoneal fluid was measured by pouring the abdominal contents into a dish after cutting open the abdomen and, after a little while, removing the viscera so that only the peritoneal fl id remained in the dish.

Gross observation at autopsy comprised the size of the lungs, the moisture of the surfaces, and the appearance of the lung surface, the latter being the most important factor The lung surface showed the following features in different conditions

In normal lungs the surface was smooth and pink

Areas of atelectasis were in most cases seen in both lower lobes, some times in the smaller lobes and occasionally locally at the lung borders. The lungs were small in cases of extensive atelectasis. The colour was bluish purple or brown, occasionally purple with white spots.

Areas of pneumonia were as a rule reddish-brown and clearly def ned.

Hyperaemia was seen in animals which had died spontaneously or after incomplete bloodletting the lung surface was dark red and blood ran profusely from the section surface.

Haemorrhages were manifested by small red dots, in a few cases spots, or rarely large areas of bleeding. In

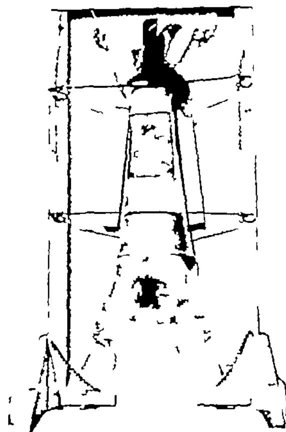


Figure 1 The position of the rabbit fixed to the table during X-ray examination The ani mal's back is enclosed in a metal shield provided with a window

son M D) examined the films without knowing from which series they were taken but being informed of the amount of pleural effusion and ascites at autopsy Radiographic changes con sistent with pulmonary oedema, and changes of other type were recorded. The extent to which the changes were conditioned by factors other than pul monary oedema was not assessed, how ever The heart size was estimated and graded as "0 + ++ and +++"

The following typical set of pictures will serve as an example

Fig 3 shows the chest film of a normal rabbit

Figs 4 5 6 and 7 show different degrees of pulmonary oedema coded as +" "++ and "+++"

Fig 8 shows pneumonia in the upper lobes of a vagotomized rabbit

Fig 9 shows atelectasis in profuse pleural effusion

Fig 10 shows large pulmonary haem orrhages

Fig 11 shows cardiac enlargement "+++" without any pulmonary change.

Gross observation at autopsy

Durlacher et at. (1950) examined the lung weight after seven different methods of killing the rabbits (bloodletting, ether anaesthesia, intravenous injection of air electrocution, rabbit punch MgSO intra venously and Nembutal intravenously) Only a slight difference in the weight of the lungs, irrespective of manner of death, was noted if the rabbits were examined immediately after death. In rabbits ex amined after lying dead for 3 hours the lung weight did not rise in those killed by bloodletting, but increased markedly after all the other methods, and most markedly after death from acute barbiturate poison ing

Injection of 275 mg of Narkotal intra venously gave in our experiments hyper aemia in 4 rabbits examined 10 minutes after death, and massive pulmonary oedema in 4 rabbits examined about 25—30 minutes after death Chest X ray examina tions made about 5 minutes post mortem (2 animals had then frothy fluid in the nose) showed no abnormalities. Pulmonary oedema can possibly arise very quickly after death but the possibility that pulmonary oedema can be present even if the radiograph is normal, should always be considered.

Bloodletting was chosen in our experiments after comparison to, for instance, electrocution and intravenous injection of ir or Narkotal. It does not seem advisable to use methods that could cause or aggravate pulmonary oedema, haemorrhages, hyperaemia, or atelectasis. On the other hand, in my experience bloodletting can be made more or less complete which may influence the autopsy findings (the blood supply in the vessel and the weight of the lungs); the preparations are also fairly time-consuming and many of our animals died spontaneously before the planned bloodletting could be started.

The following technique was used at autopsy. When breathing had ceased the trachea was ligated tightly. The chest was opened with scissors by cutting off the ensiform process and some of the lower ribs. The heart was in most cases beating at the beginning of the autopsy. The fluid in the pericardial sac was sucked out by means of a small pipette. The entire anterior portion of the chest was then cut open and removed together with the sternum and the anterior parts of the ribs. The root of the lung was ligated bilaterally including vessels and bronchi. The pleural fluid was sucked out of the pleural cavities. The heart lung preparation was freed caudally and lifted out of the chest. The stretched trachea was cut open and examined for the presence of frothy fluid. The trachea, the oesophagus, and other attachments were then severed. Each lung was cut off separately from the heart lung preparation and placed in an open beaker of thin aluminium foil. The thread around the root of the lung together with vessels and bronchial portions outside the lung were cut away. The lungs were examined macroscopically and weighed. The water content of the lung was estimated by examination of the cut surface through the lung and by judging how easily fluid could be expressed, and was coded as + ++ and +++. The left lung was used for determination of the water content. From the most markedly changed parts of the right lung, pieces were taken for microscopical examination; these were usually of hazelnut size, on a few occasions consisting of whole lobes. If no gross pulmonary changes were seen, and when microscopical examination was considered warranted, pieces were taken from the basal and hilar parts of the right lower lobe in which, empirically, the incidence of pulmonary changes is highest. The pieces were fixed in 10 % formalin. The amount of peritoneal fluid was measured by pouring the abdominal contents into a dish after cutting open the abdomen and, after a little while, removing the viscera so that only the peritoneal fluid remained in the dish.

Gross observation at autopsy comprised the size of the lungs, the moisture of the surfaces, and the appearance of the lung surface, the latter being the most important factor. The lung surface showed the following features in different conditions.

In normal lungs the surface was smooth and pink.

Areas of atelectasis were in most cases seen in both lower lobes, sometimes in the smaller lobes and occasionally locally at the lung borders. The lungs were small in cases of extensive atelectasis. The colour was bluish-purple or brown, occasionally purple with white spots.

Areas of pneumonia were as a rule reddish-brown and clearly defined.

Hyperaemia was seen in animals which had died spontaneously or after incomplete bloodletting; the lung surface was dark red and blood ran profusely from the section surface.

Haemorrhages were manifested by small red dots, in a few cases spots, or rarely large areas of bleeding. In

many cases of long survival time the colour was brownish black.

Markedly developed pulmonary oedema was easily recognisable but in *mild forms the assessment was subjective.*

Farber (1637) by direct inspection saw pulmonary oedema developing in vagotomized animals. The changes began with petechiae, which fused into larger areas with red discoloration, later found to be consolidated areas. Poulsen (206) described the lungs in excessive oedema as full and heavy with more or less extensive hyperaemia less pronounced pulmonary oedema is not directly visible but must often be suspected in the presence of fairly extensive hyperaemia, which usually precedes or accompanies oedema formation.

In the *present study the lung oedema* was graded as follows "+++" Mottled lung surface with the surface divided into numerous small diffuse spots of various colour combinations such as reddish pink, dark red to red purple to beige, bluish brown, reddish brown to white. Hilar and basal parts were occasionally wholly dark red or purple

"++ Spotted lung surface, the surfaces of the lower lobes in particular being partly covered with large spots about 1 cm in diameter unevenly and diffusely defined and most often of reddish colour occasionally of brown in rare cases white shades.

+ Dotted lung surface the surface, notably *hilary or basally being* studded over with numerous red dots or reddish brown small spots or numerous white dots seen on shining moist surfaces Lungs were rich in blood and brownish red (transition between hyperaemia and pulmonary oedema) when the variations in colour were asymmetrical and uneven and the changes were distinctly localized bilaterally in the hilar regions. In the vagotomy series, brown changes bilaterally in the upper lobes and parts of the lower lobes were graded as +

Frothy fluid in the air ways was noted by site a) the bronchi b) the trachea or c) flowing through the mouth or the orifices of the nose. As far as possible frothy fluid from pulmonary oedema was distinguished from that produced at agony or post mortem by phlegm blood, or aspirated matter Frothy fluid has been graded in other studies (e.g 170)

The *weight of the lungs* has been used by many authors in grading pulmonary changes in experimental animals (17 48 73 114 152, 169 206 218 221 232 237 256)

Durlacher et al. (73) reported a lung weight of 3.57 ± 0.21 and Alwall et al. (21) of 4 0 (3 0—5.2) g per kg body weight in bled rabbits. *Luisada (169) quoted a lung* weight per kg body weight in rabbits of 4 55 ± 0.21 g in normal animals and *13.50 ± 0.35 g in bled* animals which had received adrenaline injections. Courtice (48) found that the lung weight per kg *body weight* averaged about 4 90 g in normal rabbits, 5.50 g in rabbits with slight pulmonary oedema 6.30 g in those with moderate pulmonary oedema, and 8 10 g in those with severe pulmonary oedema. Stone & Loew (237) noted that in rabbit weighing 2.59 (2 00—3 00) kg and killed by a blow on the head the lung weight was 10.58 ± 1 41 g After a large dose of

adrenaline intravenously the lung-weight rose to 21 77 ± 7 10 g and after phenobarbitone plus adrenaline to 34.05 ± 14 49 g

Two noteworthy arguments have been advanced against the use of the lung weight as a criterion of pulmonary oedema. Durlacher et al. (1950) found that the weight of the lungs increased after death and that with some manners of death in experimental animals it could rise twofold in 3 hours. Poulsen (1954) asserted that increased lung weight could be due not only to pulmonary oedema but also to hyperaemia and pneumonia, and therefore recommended determination of the water content of the lung, since this did not increase in hyperaemia and pneumonia, whereas it did so in pulmonary oedema

Determination of the water content of the lung

The water content of the lung has been determined by desiccation (1 178, 180, 206, 213 256, nd others) Reichsman (213) found that in rabbits the water content in the left lung (the right one was used for microscopical examination) in marked pulmonary oedema veraged 85.2 (80.2—89.5) % whereas in normal rabbits or those with slight pulmonary oedema the water content was 77 1 (73.8—81.2) %. Mendenhall et al., using the freeze-drying technique, noted an verage water content of 79 6 % in the rabbit lung, 85.2 % in blood, and 92.0 % in oedema fluid drying in an oven gave almost identical results. Manery et al. (1939) reported an average w ter content of 80.2 % in the lung nd 82 5 % in the blood. Poulsen (1954) found that in mice the water content of the lung averaged 77 1 %, that of the blood 78.5 % and that of oedema fluid 94.9 %. H concluded that it seems evident that the water content of lungs howing a weight increase due exclusively t an increased accumulation of blood does not differ essentially from that of n rmal lungs. On the other hand, a weight increase caused by oedema fluid will raise considerably the water content of the lungs. He considered that the water content would hence be diagnostic in distinguishing pulmonary oedema from hyperaemia and inflammation.

According to Poulsen, freeze-drying for 48 hours maintains the lung-volume unchanged, whereas heat-drying of organic matte especially that containing blood, causes loss of volume and formation of tough membranes that prevent complete drying.

The method has been criticized by several authors. Wood & Moe (1942) considered that the water content could not be regarded as a reliable criterion of oedema, since the protein concentration in the oedema fluid varies markedly and since the results differ according to the length of drying and the temperature. Visscher et al (246) did not consider the method reliable, as the blood-cell/plasma ratio was increased in the blood present in the lung. Reichsman (213) on the other hand, found a close correlation between water content and the amount of pulmonary oedema observed by gross examination.

The following technique was used in th present study: The left lung was placed in a aluminium-foil beaker was cut into mall pieces and desiccated under infrared light (Ultra X system Groneri infrared lamp) The heat radiation from the lamp could be regulated by means of a slide resistor The desiccation was conti ued un ill two weighings at 5-minute intervals ga e the same weight Lung tissue cut in

many cases of long survival time the colour was brownish black.

Markedly developed pulmonary *oedema was easily recognisable but* in mild forms the assessment was sub jective.

Farber (1937) by direct inspection saw pulmonary oedema developing in vagotomized animals. The changes began with petechiae which fused into larger areas with red discoloration later found to be consolidated areas Poulsen (206) describ ed the lungs in excessive oedema as full and heavy with more or less extensive hyperaemia less pronounced pulmonary oedema is not directly visible but must often be suspected in the presence of fair ly extensive hyperaemia which usually *precedes or accompanies oedema forma* tion

In the present study the lung oedema was graded as follows "+++" Mott led lung surface, with the surface di vided into numerous small diffuse spots of various colour combinations such as reddish pink, dark red to red, *purple to beige, bluish brown reddish* brown to white. Hilar and basal parts were occasionally wholly dark red or purple

++" Spotted lung surface, the sur faces of the lower lobes in particular being partly covered with large spots about 1 cm in diameter unevenly and diffusely defined, and most often of reddish colour occasionally of brown in rare cases white shades.

+" *Dotted lung surface*, the sur face notably hilary or basally being studded over with numerous red dots or reddish brown *small spots or* nu merous white dots seen on shining moist surfaces. Lungs were rich in blood and brownish red *(transition* between hyperaemia and pulmonary oedema) when the variations in colour were asymmetrical and uneven and the changes were distinctly localized bi laterally in the hilar regions. In the vagotomy series, brown changes bilat erally in the upper lobes and parts of the lower lobes were graded as +"

Frothy fluid in the air-ways was noted by site a) the bronchi b) the trachea or c) flowing through the mouth or the orifices of the nose. As far as possible frothy fluid from pulmonary oedema was distinguished from that produced at agony or post mortem by phlegm blood, or aspirated matter Frothy fluid has been graded in other stud ies (e.g 170)

The *weight of the lungs* has been used by many authors in grading pulmonary changes in experimental animals (17 48 73 114 152 169 206 218 221 *232 237 256)*

Durlacher et al. (73) reported a lung *weight of* 3 57 ± 0.21 and Alwall et al. (21) of 4 0 (3 0—5.2) g per kg body weight in bled rabbits. Luisada (169) quoted a lung weight per kg body weight in rabbits of 4.55 ± 0.21 g in normal animals and 13 50 ± 0.35 g in bled animals which had received adrenaline injections. Courtice (48) *found that the lung weight per kg* body weight averaged about 4 90 g in nor mal rabbits, 5.50 g in rabbits with light pulmonary oedema, 6.30 g in those with moderate pulmonary oedema, and 8 10 g in those with severe pulmonary oedema Stone & Loew (237) noted that in rabbits *weighing 2.59 (2.00—3 00) kg and killed* by a blow on the head the lung weight wa 10.58 ± 1 41 g After a large dose of

adrenaline intravenously the lung-weight rose to 21.77 ± 7.10 g and after phenobarbitone plus adrenaline to 34.95 ± 14.49 g.

Two noteworthy arguments have been advanced against the use of the lung weight as a criterion of pulmonary oedema. Durlacher et al. (1950) found that the weight of the lungs increased after death and that with some manners of death in experimental animals it could rise twofold in 3 hours. Poulsen (1954) asserted that increased lung weight could be due not only to pulmonary oedema but also to hyperaemia and pneumonia, and therefore recommended determination of the water content of the lung, since this did not increase in hyperaemia and pneumonia, whereas it did so in pulmonary oedema.

Determination of the water content of the lung

The water content of the lung has been determined by desiccation (1, 176, 180, 206, 213, 256, and others). Reichsman (218) found that in rabbits the water content in the left lung (the right one was used for microscopical examination) in marked pulmonary oedema averaged 85.2 (80.3—89.5) % whereas in normal rabbits or those with slight pulmonary oedema the water content was 77.1 (73.8—81.3) %. Mendenhall et al. using the freeze-drying technique, noted an average water content of 79.8 % in the rabbit's lung, 83.3 % in blood, and 92.0 % in oedema fluid; drying in an oven gave almost identical results. Manery et al. (1939) reported an average water content of 80.2 % in the lung and 82.5 % in the blood. Poulsen (1954) found that in mice the water content of the lung averaged 77.1 %, that of the blood 78.5 %, and that of oedema fluid 94.9 %. He concluded that it seems evident that the water content of lungs showing a weight increase due exclusively to an increased accumulation of blood does not differ essentially from that of normal lungs. On the other hand, a weight increase caused by oedema fluid will raise considerably the water content of the lungs. He considered that the water content would hence be diagnostic in distinguishing pulmonary oedema from hyperaemia and inflammation.

According to Poulsen, freeze-drying for 48 hours maintains the lung-volume unchanged, whereas heat-drying of organic matter especially that containing blood, causes loss of volume and formation of tough membranes that prevent complete drying.

The method has been criticized by several authors. Wood & Moe (1942) considered that the water content could not be regarded as a reliable criterion of oedema since the protein concentration in the oedema fluid varies markedly and since the results differ according to the length of drying and the temperature. Visscher et al. (246) did not consider the method reliable as the blood-cell/plasma ratio was increased in the blood present in the lung. Reichsman (218) on the other hand, found a close correlation between water content and the amount of pulmonary oedema observed by gross examination.

The following technique was used in the present study. The left lung was placed in an aluminium-foil beaker, was cut into small pieces and desiccated under infrared light (Ultra X system Gronert infrared lamp). The heat radiation from the lamp could be regulated by means of a slide resistor. The desiccation was continued until two weighings at 5-minute intervals gave the same weight. Lung tissue cut in

very minute pieces dried more quickly than did less finely divided tissue, but the water content was the same in lungs from the same animal. The dry weight was cal culated as a percentage of the original lung weight. The percentage water content of the lung was assumed to be 100 minus the percentage dry weight.

In 10 rabbits the heat radiation was continued for another 30 minutes. The lung pieces did not become charred and did not lose weight. In 28 rabbits both the right and the left lung were desiccated individually the lungs showed no gross changes The average water content was 80 6 % for all the right lungs and 80 0 % for all the left lungs. Thus, the difference between the two lungs in the same animal averaged 0.5 (0—1 7) % Desiccation of blood gave very varying water contents, as thick membranes formed on the surface and prevented the blood underneath from drying The weight fell continuously during the de siccation process even after the blood had dried, because the membranes on the surface became charred. At desic cation of blood filled cut lungs char red membranes did not form and when the lungs had dried the weight did not fall in spite of continued ra diation.

In choosing the method for drying of the lung (lung tissue) to constant weight the alternatives were the tech nically simpler heat lamp method or freeze-drying (freezing at —20 C, dry ing in vacuo at —1 C for 24 hours, and thereafter drying in vacuo at room temperature) The first mention ed method has been reported to be less effective (211) The experiments de scribed below were carried out with lungs from 5 normal rabbits which had been killed by bloodletting and 5 nor mal rabbits killed by intravenous in jection of 5 ml of Narkotal solution. In the first series the lungs contained little blood and in the second series much blood.

The left lung from each animal was cut out into small pieces which were dried means of a heat lamp as describ- ed in the foregoing and the right lung was dried whole by the freeze-drying method, suspended from a thing thread, a small piece of a bronchus being kept for attachment of the thread. In the bled animals the water content of the lungs, determined by the said methods, were 80.3 (79 7—81 1) % and 79 0 (77.8 —80 7) % respectively the correspond ing figures for the animals killed by injection of Narkotal were 81.2 (81 0—81 6) % and 79 0 (77 1—80 0) %

The practical conclusions from these experiments are that highly blood fil led lungs can be adequately dried by the heat lamp technique, and that the values for the water content of the lung obtained by this method are as reliable as those obtained by the freeze drying method Therefore, the techni cally simpler procedure (the heat lamp technique) was used in my experiments It should be noted that the values were slightly higher with the heat lamp method than with the freeze-drying method the difference is slight How ever and seems to be of no practical importance as far as the experimental results and the conclusions are con cerned.

Microscopical studies of fixed sectioned and stained material have proved dis appointing in experimental acute massive oedema.

There is sometimes no stainable material in the alveoli. This result is not surprising because it is known that in early acute lung oedema of several types the oedema fluid may be nearly protein-free, as was shown by Koenig (1949) and others. The only early evidence of such lung oedema that is visible microscopically is interstitial-wall thickening because protein-free fluid in alveoli is undetectable in conventional histological preparations (Visscher et al. 246). Reichsman (1946) found that in lung oedema after bilateral vagotomy in mice the microscopical picture usually confirmed the gross examination with the exception that small and sometimes even moderate amounts of lung oedema were difficult to demonstrate under the microscope. Durlacher et al. showed that in rabbits which had been dead for 3 hours or more, alveolar oedema developed after death.

The lungs of 520 rabbits were studied microscopically. The slides were examined by Ass. Prof. N. O. Berg at the Institute of Pathology. The following typical features were noted.

Normal picture (Figs. 12 and 13).

Bleeding (Figs. 14 and 15). Focal haemorrhages were a characteristic finding in many series. The haemorrhages varied in number and size and involved from a few to several hundred alveoli.

Hyperaemia (Fig. 16) was relatively rare in the bled animals, whereas all stages, from insignificant to massive hyperaemia in all the capillaries, were seen in the animals that died spontaneously.

Interstitial oedema (Fig. 17) occurred in the form of distension of the periarterial tissue in the lung.

Bronchiolitis (Figs. 18 and 19) with more or less marked destruction of the epithelium in the bronchioles but not in the larger bronchi, was seen in the 'Narkotal' series.

Purulent bronchitis and stagnation of secretion were common findings in some series and were probably due to aspiration.

Atelectasis was one of the most common changes. In many cases the changes involved only a small part of the lung.

Pneumonia was noted mainly in vagotomized animals. A slight increase of leucocytes in the alveolar walls without any other signs of pneumonia was demonstrated in the adrenaline series.

Thickening of the alveolar septa of varying degree was seen in animals with hyperaemia, pulmonary oedema, or decreased content of air in the alveoli, but this finding was usually not recorded in this investigation, since the thickness of the septa often varied in the same animal.

Alveolar oedema (Figs. 20, 21, 22, 23, 24 and 25) was seen as precipitated albumin in the alveoli, in typical cases containing a few red blood-cells and alveolar phagocytes. The degree of alveolar oedema was coded as +, ++ and +++.

The lung-volume was determined in some 30 rabbits by immersion of the right lung in a beaker filled with water. The overflow which discharged through a spout, was collected and weighed. The trachea and the hilum were tied so as to prevent

very minute pieces dried more quickly than did less finely divided tissue, but the water content was the same in lungs from the same animal. The dry weight was calculated as a percentage of the original lung weight. The percentage water content of the lung was assumed to be 100 minus the percentage dry weight.

In 10 rabbits the heat radiation was continued for another 30 minutes. The lung pieces did not become charred and did not lose weight. In 28 rabbits both the right and the left lung were desiccated individually: the lungs showed no gross changes. The average water content was 80.6 % for all the right lungs and 80.6 % for all the left lungs. Thus, the difference between the two lungs in the same animal averaged 0.5 (0—1.7) %. Desiccation of blood gave very varying water contents as thick membranes formed on the surface and prevented the blood underneath from drying. The weight fell continuously during the desiccation process even after the blood had dried, because the membranes on the surface became charred. At desiccation of blood filled cut lungs charred membranes did not form, and when the lungs had dried the weight did not fall in spite of continued radiation.

In choosing the method for drying of the lung (lung tissue) to constant weight the alternatives were the technically simpler heat lamp method or freeze-drying (freezing at −20 C, drying in vacuo at −1 C for 24 hours, and thereafter drying in vacuo at room temperature). The first mentioned method has been reported to be less effective (211). The experiments described below were carried out with lungs from 5 normal rabbits which had been killed by bloodletting and 5 normal rabbits killed by intravenous injection of 5 ml of Narkotal solution. In the first series the lungs contained little blood and in the second series much blood.

The left lung from each animal was cut out into small pieces which were dried means of a heat lamp as described in the foregoing, and the right lung was dried whole by the freeze-drying method, suspended from a thing thread, a small piece of a bronchus being kept for attachment of the thread. In the bled animals the water content of the lungs, determined by the said methods, were 80.3 (79.7—81.1) % and 79.0 (77.8—80.7) % respectively; the corresponding figures for the animals killed by injection of Narkotal were 81.2 (81.0—81.6) % and 79.0 (77.1—80.0) %.

The practical conclusions from these experiments are that highly blood filled lungs can be adequately dried by the heat lamp technique, and that the values for the water content of the lung obtained by this method are as reliable as those obtained by the freeze-drying method. Therefore, the technically simpler procedure (the heat lamp technique) was used in my experiments. It should be noted that the values were slightly higher with the heat lamp method than with the freeze drying method; the difference is slight, however, and seems to be of no practical importance as far as the experimental results and the conclusions are concerned.

CHAPTER 8

Control material

The control material consisted of 50 normal rabbits and 78 rabbits whose ureters were tied and which were killed by bloodletting 0 1 and 1 2, 3 or 4 days, respectively after the operation. The lungs were also studied in rabbits which after ligation of the ureters were killed by other methods or allowed to die spontaneously in their uraemia. The ligation of the ureters was made bilaterally above the pubes at the bladder under anaesthesia with Xylocain®

Examination of the living animals

Appearance and behaviour In most cases no symptoms were noted on the first few days after ureteral ligation Thereafter the animals became increasingly drowsy and rarely took food o drink. In those with severe uraemia trembling twitchings, and unsteady gait were common features. Diarrhoea occurred in many cases. Marked muscle weakness intercurred the last few hours before death the nimals lay down on their sides and did not move much Many of them died unnoticeably

The animals lost weight, on an average about 2 % when the bladder was emptied during the ureteral ligation, and thereafter about 1 % per day A few animals gained weight on the first days after their ureters had been tied, whereas in others diarrhoea led to a weight loss of 4 % or more per day The average weight loss in uraemic rabbits is probably explained by inappetence (food and water) reduction of the gastrointestinal contents, tissue breakdown, and loss of fluid through diarrhoea.

Laboratory data In 37 rabbits non protein nitrogen (N P N) in the blood (determined by Rappaport s method) rose after the ureteral ligation by about 70 (40—120) mg per 100 ml per day from about 45 (28—68) mg per 100 ml before the operation (Table 1) in the rest of the rabbits, N P.N was determined only at the bloodletting. The highest recorded value in all the series was 420 mg per 100 ml in a rabbit which was killed by bloodletting after 4 days. In most cases of spontaneous death in uraemia the N P N level was about 300 mg per 100 ml. Serum potassium (determined by flame photometry) increased gradually to high values (Table 1) after 4 days of anuria the mean value was 10 0 mEq per litre.

Respiratory rate was checked for 4

air from leaving the lung during dissection. Before the measuring procedure, the lung root was cut off close to the thread, a pair of heavy forceps was attached to the thread and the forceps and the lung were placed in the beaker

The *diagnosis of pulmonary oedema* was considered established in the present study if at least three of the following examinations were positive Chest X ray gross examination, estimation of frothy fluid in the air ways, measurements of lung weight determination of the water content in the lungs, and microscopical studies In the vagotomy series, the three criteria of pulmonary oedema should include microscopical alveolar oedema or a water content above the upper limit of normal range. The extent to which the individual criteria vary under experimental conditions (see Chapters 6—9) will be discussed in Chapter 10

CHAPTER 5

Control material

The control material consisted of 69 normal rabbits and 78 rabbits whose ureters were tied and which were killed by bloodletting 0 1 and 1 2 3, or 4 days, respectively after the operation. The lungs were also studied in rabbits which after ligation of the ureters were killed by other methods or allowed to die spontaneously in their uraemia. The ligation of the ureters was made bilaterally above the pubes at the bladder under anaesthesia with Xylocain®

Examination of the living animals

Appearance and behaviour In most cases no symptoms were noted on the first few days after ureteral ligation. Thereafter the animals became increasingly drowsy and rarely took food or drink. In those with severe uraemia trembling, twitchings, and unsteady gait were common features. Diarrhoea occurred in many cases. Marked muscle weakness intercurred the last few hours before death the animals lay down on their sides and did not move much. Many of them died unnoticeably

Th animals lost weight on an average about 2 % when the bladder was emptied during the ureteral ligation and thereafter about 1 % per day A few animals gained weight on the first days after their ureters had been tied, whereas in others diarrhoea led to a weight loss of 4 % or more per day The average weight loss in uraemic rabbits is probably explained by inappetence (food and water) reduction of the gastrointestinal contents, tissue breakdown, and loss of fluid through diarrhoea.

Laboratory data In 37 rabbits non protein nitrogen (N.P.N.) in the blood (determined by Rappaport s method) rose after the ureteral ligation by about 70 (40—120) mg per 100 ml per day from about 45 (28—68) mg per 100 ml before the operation (Table 1) in the rest of the rabbits, N P N was determined only at the bloodletting. The highest recorded value in all the series was 426 mg per 100 ml in a rabbit which was killed by bloodletting after 4 days. In most cases of spontaneous death in uraemia the N.P.N level was about 300 mg per 100 ml. Serum-potassium (determined by flame photometry) increased gradually to high values (Table 1) after 4 days of anuria the mean value was 10.0 mEq per litre.

Respiratory rate was checked for 4

Table 1 *N.P.N and serum potassium in rabbits 0—4 days after bilateral ligation of their ureters*

Anuria days	N P N mg per 100 ml	Number of rabbits	Serum potassium mEq/l	Number of rabbits
0	45 (28—68)	37		
1	98 (66—128)	37		
2	175 (120—223)	34	6.8 (4.9—9.3)	18
3	275 (190—405)	32	8.9 (6.6—10.2)	8
4	330 (244—426)	13	10 0 (9 1—12.5)	7

days in 8 normal rabbits in connection with *for instance, transportation* weighing and electrocardiographic examination The respiratory rate averaged 122 (72—220) per minute. When the animals were at rest and undisturbed the rate fell when they were agitated it rose to more than double the normal value The highest respiratory rate was 430 per minute, measured by means of a thermistor in front of the nostrils.

Table 2 Respiratory rate was measured daily for 3 days in 51 rabbits whose ureters were tied The average rate fell with increasing uraemia as a rule most markedly at an N.P N level exceeding *200* mg per *100* ml *In most cases respiration became gradu*ally slower and was finally suspended

Table 2 *Mean respiratory rate per minute for 51 rabbits 0—3 days after ligation of their ureters and estimated mean value for N.P.N*

Anuria days	Respiratory rate per minute mean and range	N P N mg per 100 ml (estimated mean value)
0	153 (58—428)	45
1	127 (38—238)	101
2	09 (33—180)	171
3	79 (33—140)	275

Table 3 *Heart rate per minute and estimated mean value for N.P.N in 51 rabbits 0—3 days after ligation of their ureters*

Anuria days	Heart rate per minute mean and range	N P N mg per 100 ml (estimated mean value)
0	218 (154—294)	45
1	224 (168—278)	101
2	219 (139—273)	171
3	230 (151—316)	75

The heart rate, measured by electrocardiography showed great variations. *In 233 normal rabbits the average rate* was 230 (125—368) beats per minute In the 51 rabbits with tied ureters the rate altered little during 3 days of uraemia finally bradycardia was noted however (Table 3)

Electrocardiographic abnormalities became more frequent with increasing uraemia Fig 2 shows the most characteristic change. The positive T wave became increasingly flat and finally changed to negative. Broadening and deformation of the QRS complex, extrasystoles, arrhythmia, and bradycardia were noted in the final stage Table 4 shows that after 3 days the T waves were *absent in 10 %* and negative in 25 % In 15 rabbits with anuria for 3—4 days there was poor correla

Table 4 *Occurrence of T-wave abnormalities in the electrocardiograms and estimated mean value for N.P.N in 51 rabbits 0—3 days after ligation of their ureters*

Anuria day	Number of rabbits with T waves positive	absent	negative	N P N mg per 100 ml (estimated mean value)
0	51	0	0	45
1	51	0	0	101
2	43	4	4	171
3	33	5	13	275

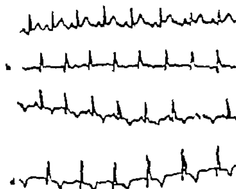


Figure 2 Electrocardiogram (lead I) in an animal with typical changes. Examination in this case carried out) before, b) 2 days after c) 3 days after d) 4 days after ligation of the ureters.

tion between the serum potassium concentration and the electrocardiographic pattern. Accordingly the electrocardiogram was normal in 2 animals with a potassium level of 10 1 mEq per litre, whereas it was greatly abnormal in another animal with a potassium concentration of 6.6 mEq per litre.

It was found that the clinical pictures, respiratory rates, and electrocardiograms of animals which had survived the first few days of anuria served as a relatively reliable basis for the estimation of remaining survival time.

Table 5 *Occurrence f radiographic pulmonary hanges coded as "0" and + more severe changes did not occur) in 381 anuri rabbit 1—4 day aft ligation f the ureters*

Anuria day	Pulmonary changes 0	+
1	46	0
2	222	3
3	96	3
4	9	0

Chest radiography In all the rabbits of the experimental series chest X ray before ligation of the ureters showed normal lungs and normal heart size. Table 5 After ligation of the ureters slight pulmonary changes were seen within 3 days in 3 % of the animals. — Table 6 Enlarged heart shadow In most cases a slight increase, was noted in about 15 % of the severely uraemic animals.

Table 6. *Occurrence of radiographically enlarged heart coded as "0" + ++ and +++ in 381 anuric rabbits 1—4 days aft r ligation f their uret rs*

Anuria day	Cardiac enlargement 0	+	++	+++
1	44	2	0	0
2	200	16	0	0
3	86	11	3	1
4	6	2	1	0

Examination immediately after death of bled animals

Gross observation at autopsy In all the normal rabbits the lung surfaces were smooth and pink. Among the rabbits with tied ureters no dots or spots were seen in 20 after less than 2 days of anuria. A few red dots, in rare cases small spots, were noted on the lung surfaces in 5 out of 28 rabbits (18 %) after 2 days of anuria, in 6 out of 20 rabbits (30 %) after 3 days of anuria and in 2 out of 10 (20 %) after 4 days of anuria. In severe uraemia the colour of the dots was more brown than red. In 2 severely uraemic animals the lungs were bluish pink despite the absence of a large amount of pleural effusion. In one of the latter there were

Table 1 *N.P.N and serum potassium in rabbits 0—4 days after bilateral ligation of their ureters*

Anuria days	N.P.N mg per 100 ml	Number of rabbits	Serum potassium mEq/l	Number of rabbits
0	45 (28—68)	37		
1	98 (66—128)	37		
2	175 (120—223)	34	5.8 (4.9—9.3)	18
3	275 (199—403)	32	8.9 (6.6—10.2)	8
4	350 (244—426)	13	10.0 (9.1—12.5)	7

days in 8 normal rabbits in connection with for instance, transportation weighing and electrocardiographic examination. The respiratory rate averaged 122 (72—220) per minute When the animals were at rest and undisturbed, the rate fell when they were agitated it rose to more than double the normal value The highest respiratory rate was 430 per minute, measured by means of a thermistor in front of the nostrils

Table 2 Respiratory rate was measured daily for 3 days in 51 rabbits whose ureters were tied The average rate fell with increasing uraemia as a rule most markedly at an N P N level exceeding 200 mg per 100 ml. In most cases respiration became gradually slower and was finally suspended.

Table 2 *Mean respiratory rate per minute for 51 rabbits 0—3 days after ligation of their ureters and estimated mean value for N.P.N*

Anuria day	Respiratory rate per minute mean and range	N P N mg per 100 ml (estimated mean value)
0	153 (56—428)	45
1	127 (86—238)	101
2	99 (33—180)	171
3	79 (33—140)	275

Table 3 *Heart rate per minute and estimated mean value for N.P.N in 51 rabbits 0—3 days after ligation of their ureters*

Anuria day	Heart-rate per minute mean and range	N.P.N mg per 100 ml (estimated mean value)
0	218 (154—204)	45
1	224 (168—278)	101
2	219 (130—273)	171
3	230 (151—310)	275

The heart rate, measured by electrocardiography showed great variations In 233 normal rabbits the average rate was 230 (125—308) beats per minute In the 51 rabbits with tied ureters the rate altered little during 3 days of uraemia finally bradycardia was noted, however (Table 3)

Electrocardiographic abnormalities became more frequent with increasing uraemia Fig 2 shows the most characteristic change. The positive T wave became increasingly flat and finally changed to negative. Broadening and deformation of the QRS complex extrasystoles arrhythmia and bradycardia were noted in the final stage Table 4 shows that after 3 days the T waves were absent in 10 % and negative in 25 % In 15 rabbits with anuria for 3—4 days there was poor correla

Table 4 *Occurrence of T-wave abnormalities in the electrocardiograms and estimated mean value for N.P.N in 51 rabbits 0—3 days after ligation of their ureters*

Anuria day	Number of rabbits with T waves positive	absent	negative	N.P.N mg per 100 ml (estimated mean value)
0	51	0	0	45
1	51	0	0	101
2	43	4	4	171
3	33	5	13	275

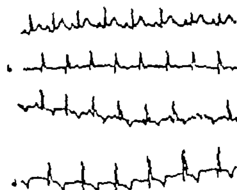


Figure 2. Electrocardiograms (lead I) in an animal with typical changes. Examination in this case carried out.) before, b) 2 days after) 3 days after d) 4 days after ligation of the ureters

tion between the serum potassium concentration and the electrocardiographic pattern. Accordingly the electrocardiogram was normal in 2 animals with a potassium level of 10 1 mEq per litre, whereas it was greatly abnormal in another animal with a potassium concentration of 6.6 mEq per litre.

It was found that the clinical pictures, respiratory rates, and electrocardiograms of animals which had survived the first few days of anuria served as a relatively reliable basis for the estimation of remaining survival time.

Table 5 *Occurrence f radiographic pulmonary changes coded as "0" and + more sever hanges did not occur) in 281 anuric abbit 1—4 day aft ligation f their uret rs*

Anuria days	Pulmonary changes 0	+
1	48	0
2	122	3
3	96	3
4	9	0

Chest radiography In all the rabbits of the experimental series chest X ray before ligation of the ureters showed normal lungs and normal heart size, Table 5 After ligation of the ureters slight pulmonary changes were seen within 3 days in 3 % of the animals. — Table 6 Enlarged heart shadow in most cases a slight increase, was noted in about 15 % of the severely uraemic animals.

Tabl 6. *Occurrence f radiographically enlarged heart coded as "0" + ++ and +++ (351 anuric rabbits 1—4 days aft r ligati n of their ureters*

Anuria days	Cardiac enlargement 0	+	++	+++
1	44	2	0	0
2	200	16	6	0
3	88	11	3	1
4	6	3	1	0

Examination immediately after death of bled animals

Gross observation at autopsy In all the normal rabbits the lung surfaces were smooth and pink. Am ng the rabbits with tied ureters no dots or spots were seen in 20 after less than 2 days of anuria. A few red dots, in rare cases small spots, were noted on the lung surfaces in 5 out of 28 rabbits (18 %) after 2 days of anuria, in 6 out of 20 rabbits (30 %) after 3 days of anuria, and in 2 out of 10 (20 %) after 4 days of anuria. In severe uraemia the colour of the dots was more brown than red. In 2 severely uraemic animals the lungs were bluish pink despite the absence of a large amount of pleural effusion. In one of the latter there were

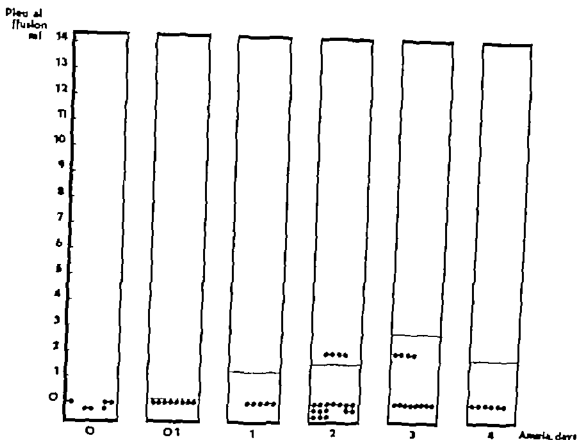


Diagram 1 Pleural effusion in normal and uraemic rabbits with anuria for 0.1 1 2, 3 and 4 days, respectively

small brown spots in the bluish red lungs.

Pleural effusion and ascites were not seen in normal rabbits, excepting 2 which had 2 ml of pleural fluid each Most of the severely uraemic rabbits had only slight pleural effusion (Diagram 1) Among rabbits with anuria for at least 2 days pleural effusion was noted in about 45 % the amount being 3 (1—14) ml.

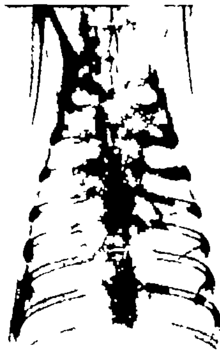
The amount of ascitic fluid increased markedly with increasing uraemia (Diagram 2) After 3 or 4 days of uraemia 30 rabbits had, on an average, 108 (?—355) ml of ascitic fluid. Thus, at increasing uraemia in rabbits with tied ureters, *there was a shifting of* fluid from the tissues to the abdominal cavity possibly because of the higher *osmolality in the latter (the NPN* level in the ascitic fluid was, on an average, 10 % higher than in blood)

Pericardial effusion occurred in all the rabbits the normal ones as well The amount was difficult to measure but in normal rabbits it could be estimated at 0.5 (0 1—1.5) ml. In severe uraemia the amount seemed to increase to about 2 ml the highest amount measured was 4 ml.

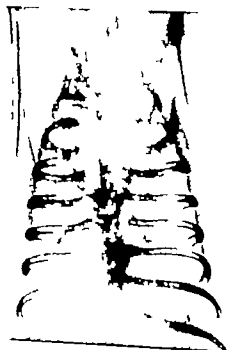
Frothy fluid in the air ways was not found, either in normal or in uraemic controls



3



4



5

Figure 3 A normal rabbit

Figure 4 Pulmonary changes coded as + (light pulmonary oedema)

Figure 5 Pulmonary changes coded ++ (moderate pulmonary oedema)

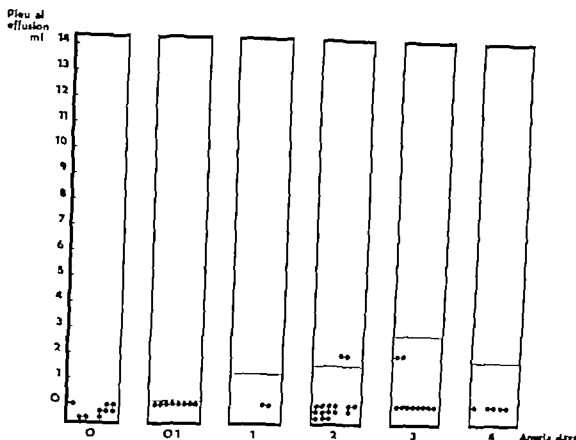


Diagram 1 Pleural effusion in normal and uraemic rabbits with anuria for 0.1 1 2, 3 and 4 days respectively

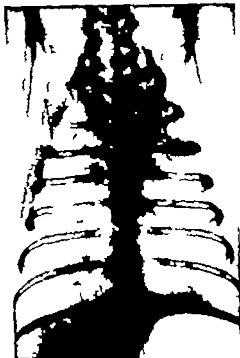
small brown spots in the bluish red lungs

Pleural effusion and ascites were not seen in normal rabbits, excepting 2 which had 2 ml of pleural fluid each Most of the severely uraemic rabbits had only slight pleural effusion (Diagram 1) Among rabbits with anuria for at least 2 days pleural effusion was noted in about 45 % the amount being 3 (1—14) ml.

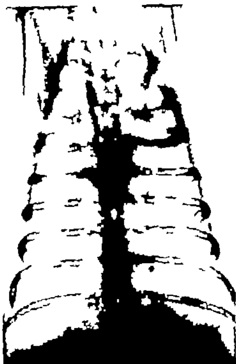
The amount of ascitic fluid increased markedly with increasing uraemia (Diagram 2) After 3 or 4 days of uraemia 30 rabbits had, on an average 108 (2—355) ml of ascitic fluid. Thus, at increasing uraemia in rabbits with tied ureters, there was a shifting of fluid from the tissues to the abdominal cavity possibly because of the higher osmolality in the latter (the NPN level in the ascitic fluid was on an average, 10 % higher than in blood)

Pericardial effusion occurred in all the rabbits, the normal ones as well The amount was difficult to measure but in normal rabbits it could be estimated at 0.5 (0 1—1.5) ml In severe uraemia the amount seemed to increase to about 2 ml the highest amount measured was 4 ml

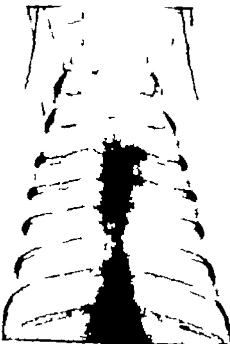
Frothy fluid in the air ways was not found, either in normal or in uraemic controls.



9



10



11

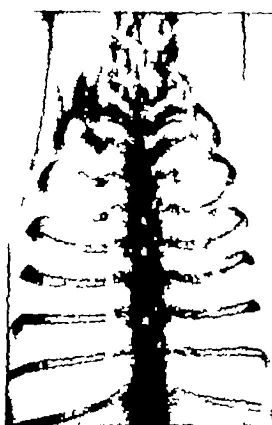
Figure 9 Atelectasis in case of profuse pleural effusion

Figure 10 Bleeding in the lung parenchyma following infusion of dextra

Figure 11 Cardiac enlargement ("+++") without pulmonary changes



6



7



8

Figure 6. Pulmonary hanges coded as +++ (severe pulmonary oedema)
Figure 7 Pulmonary changes coded a +++ (severe pulmonary oed ma)
Figure 8. Pneumonia in a vagotomized rabbit.



Figure 14 Minor haemorrhage in the lungs of uraemic rabbit. Haematoxylin-eosin. ×63.



Figure 15. Profuse haemorrhages in the lungs without alveolar oedema in rabbit with severe pulmonary oedema following infusion of dextran. Haematoxylin eosin. ×400.

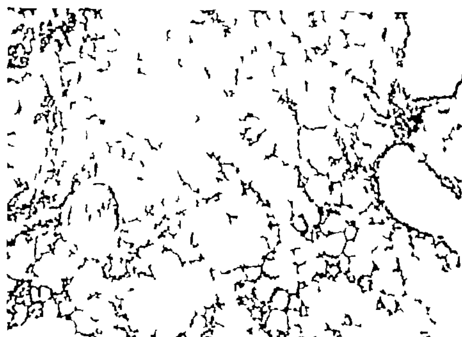


Figure 12 Normal picture of the lungs of a highly uraemic rabbit Note the strel hed al eolar wall and rounded lveoles. Haemat xylin-eosin. ×63.



Figu 13. Normal picture of the lungs of highl raemic rabbit. Not the tr t hed lveolar wall nd rounded al eoles Haemato li -eosin. ×100

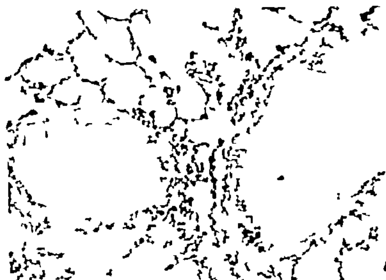


Figure 18. Bronchiolitis in raemic rabbit anaesthetized with "N rkotal" Haematoxylin-eosin ×100.

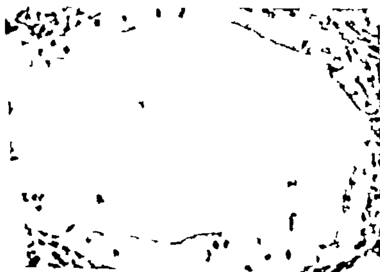


Figure 19. Bronchiolitis in uraemic rabbit naesthetized with "Narkotal" Haematoxylin-eosin. ×400.



Figure 16 Marked hypera mia without alveolar oedema in a rabbit with se ere pulmonary oedema foll wing injection of adrenaline Haematoxy lin-eosin. ×100



Fig 17 Periarterial int rstitial oedema in a rabbit without pulmonary oed ma f ll wing injecti n of adrenali there are lso minor ha mor hages. H matoxylin eosin. ×25

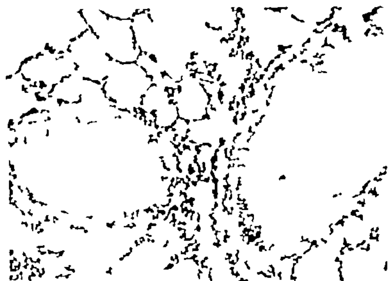


Figure 18. Bronchiolitis i[illegible] uraemic rabbit anaesthetized with "Narkotal"
Haematoxylin-eosin ×100.



Figure 19. Bronchiolitis in uraemic rabbit anaesthetized with "Narkotal"
Haematoxylin-eosin. ×400.



Figur 20 Alveolar oed ma coded as +++ in a ral bit after injection of a lrenaline Ha matoxylin eosin. ×160

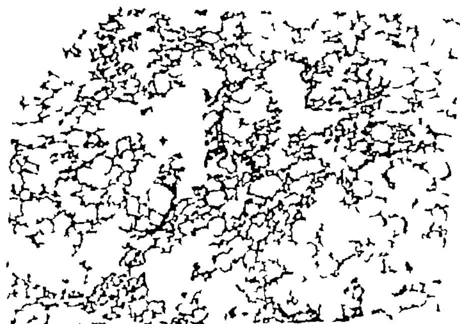


Figure 21 Alveolar oedema h re and th re listl l coded ++ in a g tomized ral bit H mato ylin-eosin. ×60

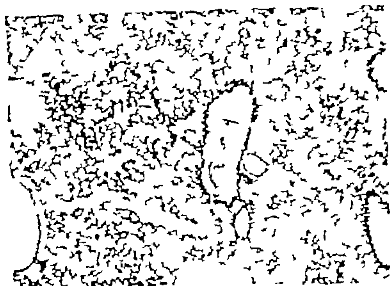


Figure [illegible] Focal fibrinous ("stringy") alveolar oedema coded as ++ in rabbit after "Narkotal" anaesthesia. Haematoxylin and eosin ×[illegible]

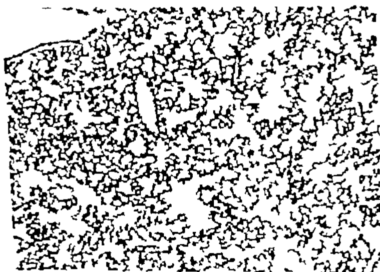


Figure [illegible] Generalized thin fibrinous ("stringy") alveolar oedema coded ++ in rabbit after "Narkotal" anaesthesia and overhydration with Ringer solution. Haematoxylin-eosin. ×[illegible]



Figure ?0 Al colar oed ma coded as +++ in a rabbit after *injection* of adrenaline Haematoxylin-eosin. ×100



Figure 1 Al colar oed ma, he and there di tinct oded a ++ in a agotomized rabbit. Ha mat yli -eo in. ×63

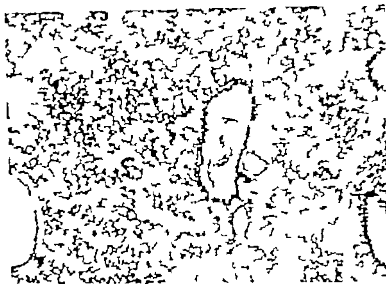


Figure 22. Focal fibrinous (alr(p)) lveola oedema coded as ++ in rabbit after "Narkotal" naesthesia. H emat xylin-eosin. ×63.

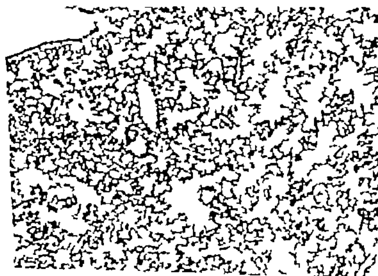


Figure 23. Generalized thin fibrinous (" fringy") alveola oedema coded ++ in rabbit after "Narkotal" naesthesia nd overhydration ith Ringer solution. Haematoxylin-eosin. ×63

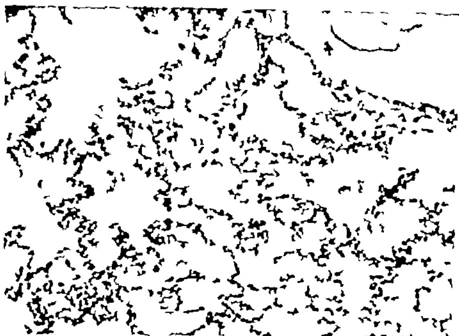
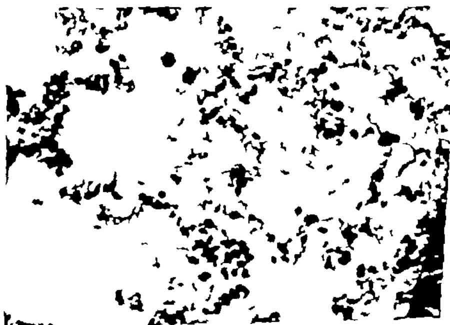


Figure 24 Generalized thin fibrinous (stringy") alveolar oedema coded as ++ In a rabbit after Narkotal anaesthesia and overhydration with Ringer s soluti n Haemato ylin-eosin ×100



Figu 25 Al eolar oedema coded as + In a rabbit after o erhydration (nfusion of d xtran I tl) H em toxylin-eosin. ×100

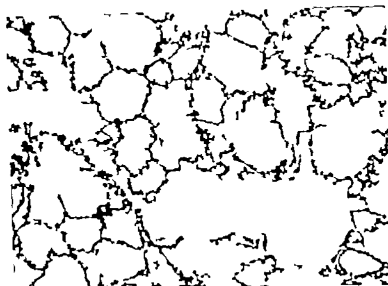


Figure 26 Insignifica t microscopical pulmonary changes in an over hydrated vagotomized rabbit, in spite of four other criteria of marked pulmonary oedema. Note the plicated al eolar walls. Haematoxylin-eosin. ×56.

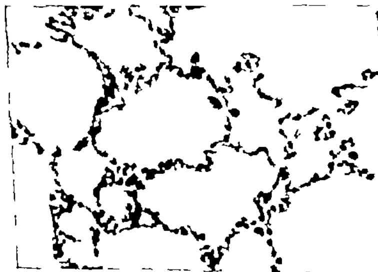


Figure 27 I significant microscopical pulmonary images in an over hydrated agotomized rabbit, in spite of four other criteria of marked pulmonary oedema. Note the plicated alveola walls. Haematoxylin-eosin. ×400.

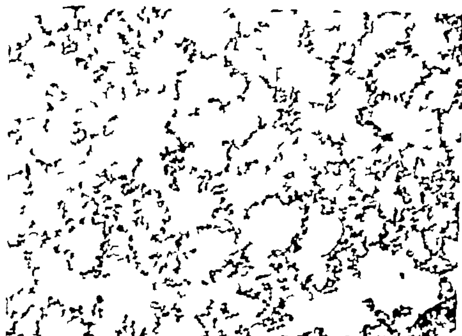


Figure 28 Postmortal pulmonary oedema. Rounded air bubbles are seen here and there in the oedema fluid. Haem to ylin-eosin ×160

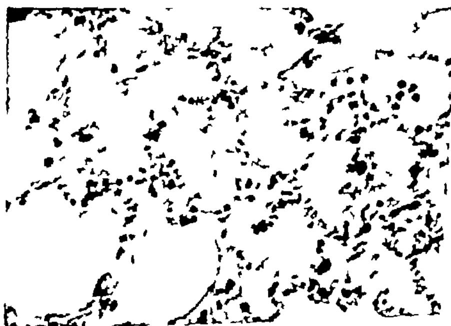


Figure 29 P stmortal pulmonary oed ma. R unded air bubbles re seen here nd there in th oedema fl id Ha mato ylin-eosin. ×400.

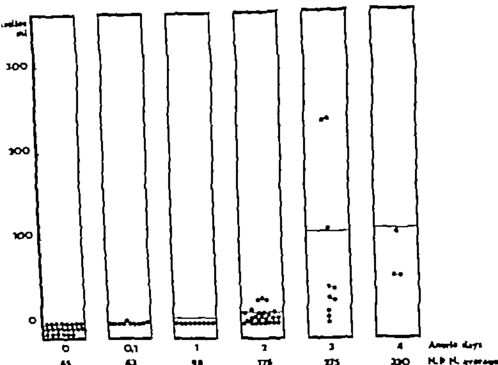


Diagram 2. Amounts of ascitic fluid in normal and uraemic rabbits with anuria for 0.1, 1, 2, 3, and 4 days, respectively.

Lung weight. (a) Relation between lung weight and body weight (Diagram 3). The average weight of the lungs of 59 bled rabbits (body weight 1.38—3.87 kg) was 10.26 g ±23.5 % or expressed as lung weight per kg body weight, 3.93 g ±14.7 %. It will be seen from the diagram that the relative value for the lung weight per kg body weight falls as the body weight increases. The animals in an experimental series should therefore have about the same weight.

Table 7 shows that in 22 bled normal rabbits weighing 2.20—2.40 kg, the lung weight was 4.17 ±0.53 g per kg body weight or expressed as normal value with 2 / sigma 2.85—5.49 g per kg body weight.

(b) Relation between lung weight and degree of uraemia (Tables 7 and 8 and Diagram 4). The lung weight of animals which were bled to death at various degrees of uraemia does not differ from that in normal animals, except in a few cases. For 78 uraemic animals the mean value was 4.25 ±0.45 g per kg body weight. The highest value, 5.07 g per kg body weight, was recorded for a rabbit with numerous small haemorrhages in the lungs.

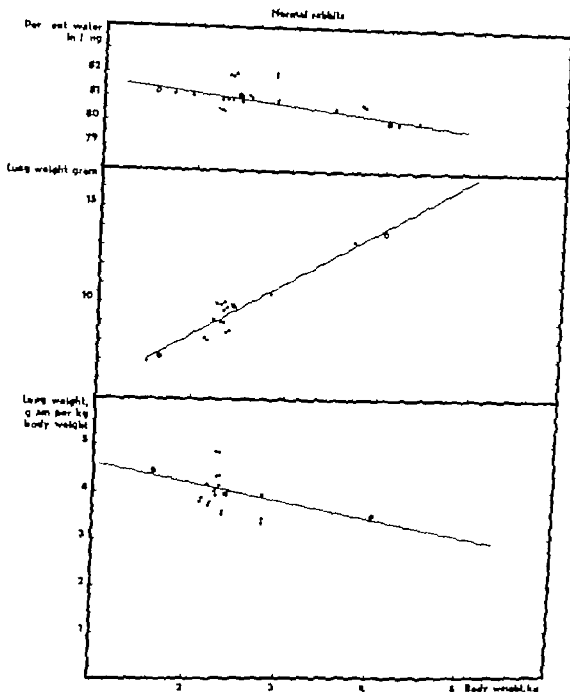


Diagram 3. Water content in the lung, lung weight, and lung weight per kg body weight in normal rabbits weighing 1.38—5.87 kg

The water content of the lung (a) Relation between the water content of the lung and the body weight (Diagram 3) The water content of the left lung expressed as a percentage of the lung weight varied in 59 bled normal rabbits (weighing 1.38—5.87 kg) between 78.4 and 82.8 % The lungs of

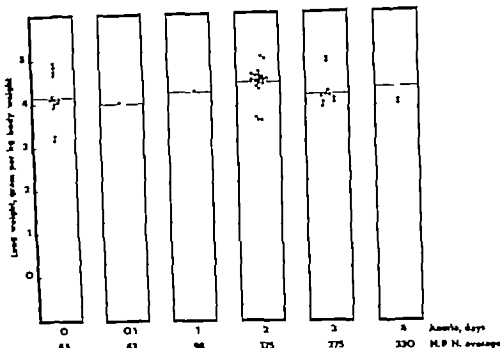


Diagram 4. Lung-weight of normal and uraemic rabbits with anuria for 0.1, 1, 2, 3, and 4 days, respectively

young animals with a low body weight had a slightly higher water content than those of older animals with a higher body weight. In 22 bled normal rabbits (weighing 2.20—2.49 kg) the water content of the left lung was 81.0 ±0.9 % or expressed as normal value with 2 / sigma, 78.7—83.3 % (Table 7).

(b) Relationship between the water content of the lungs and the degree of uraemia (Diagram 5 and Table 7). The water content of the lungs in uraemic animals was within the normal range

Table 7. *Lung-weight in g per kg body-weight, water content as a percentage, number of normal animals, bled uraemic non-overhydrated animals, overhydrated animals and spontaneously dead non-rigid uraemic animals. The values are means with 2 × standard deviation. The animal weight at the beginning of the experiment ranged from 2.20 to 2.49 kg*

	Lung-weight g per kg body-weight	Water content in the lung	Number of rabbits
Bled, normal	4.17 (2.85—5.49)	81.0 (78.7—83.3)	22
Bled, uraemic	4.25 (3.13—5.35)	80.3 (77.1—83.8)	78
Bled, uraemic, overhydrated	4.39 (3.10—5.67)	82.2 (79.2—85.2)	62
Spontaneously dead, uraemic	4.98 (3.38—6.58)	80.9 (77.1—84.7)	30

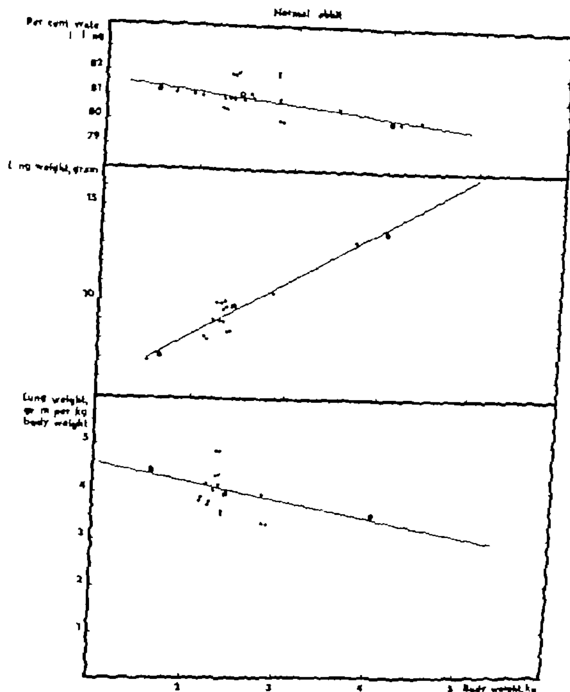


Diagram 3. W t r content in th lung, lung weight, and lung weight per kg body weight in normal rabbits weighing 1.38—5.87 kg.

The water content of the lung (a) Relation between the water content of the lung and the body weight (Diagram 3) The water content of the left lung expressed as a percentage of the lung weight varied in 59 bled normal rabbits (weighing 1.38—5.87 kg) between 78.4 and 82.8 % The lungs of

Table 9 Lung-weight in g per kg body-weight, water content as a percentage, the colour of the lung surface at gross observation post mortem, and microscopical findings in the 6 rabbits which had the highest lung-weights among 30 spontaneously dead non-rigid rabbits

Lung-weight g per kg body-weight	Water content in lung %	Colour of lung surface at gross examination	Microscopical findings	Autopsy minutes after death
6.76	82.1	Dark pink	Hyperaemia, small area of atelectasis	20?
6.65	83.2	Dark pink	Hyperaemia, small areas of atelectasis, insignificant alveolar oedema	90
6.24	84.6	Dark red	Hyperaemia, insignificant alveolar oedema	25
6.82	79.6	Purple-dark-pink	Hyperaemia, small areas of atelectasis, insignificant alveolar oedema	80
6.72	82.9	Purple pink	Hyperaemia, small area of atelectasis	90
6.44	82.1	Dark pink	Slight hyperaemia	10

ter 3 or 4 days, the picture was normal (Figs. 12 and 13). Three of the rabbits had focal haemorrhages (Fig. 14) which at the gross examination were seen as red spots on the lung surfaces. In 1 rabbit with bluish pink lungs, atelectasis was noted. Bronchiolitis and interstitial oedema were not demonstrated.

Examination after death of animals dying spontaneously without rigor mortis

39 rabbits died spontaneously in their uraemia 81 (45—147) hours after ureteral ligation, at a mean NPN level of 333 (205—444) mg per 100 ml, and were examined while their bodies were still warm and soft. a) 12 rabbits were examined within 10 minutes of death; they had pink lung surfaces without pulmonary changes, b) 16 rabbits were examined within 10—80 minutes, and c) 11 within 90 minutes of death.

Gross examination. The colour of the lung surfaces varied between pale pink, bluish-purple pink, pale red, dark red, or intermediate shades. It will be seen from Table 9 that only a few animals had dark red heavy lungs. Red or brown dots were present as frequently as in the bled animals. Spotted or mottled surfaces were not seen.

Frothy fluid in the air ways was not demonstrated in any animal.

Lung weight. (a) In 12 animals *examined within 10 minutes of death* the mean lung weight was 4.57 (3.05—5.44) g per kg body weight. In 16 examined within 10—80 minutes of

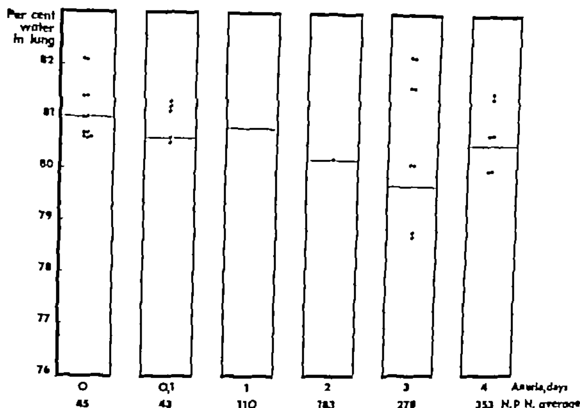


Diagram 5 Wat r content in the lungs of normal and uremic rabbits with anuria f r 0.1 1 2, 3 and 4 days, respectively

Table 8 *Lung-weight in g and g per kg body weight in bled normal animals and animals which had been anuric for 0 1—4 days before bloodletting The animals weights at the beginning of the experiment ranged from 2.20 to 2 49 kg*

	Number of rabbits	Anuria d ys	L ng-weight g mea	Lung weight g per kg body w ight
Normal	22	0	9 70	4 17
Uraemic	10	0 1	9.23	4.06
	10	1	9.80	4.36
	28	2	10 40	4.02
	"0	3	9 47	4.27
	10	4	9 79	4.68
Mean	8		9 84	4 42

in 85 % and below the lower limit for normal in 15 % The mean water con tent was 80.3 ±1.3 % that is, slightly lower in the uraemic animals than in normals. The difference is significant. Rabbits with anuria for 4 days had a normal water content in their lungs, which suggests that uraemia in itself does not lead to a low content of water in the lungs but that this is due to other factors True fluid depletion can be caused by for instance diarrhoea and relative depletion by collection of large amounts of ascitic fluid.

Microscopical examination of the lung tissue In 12 out of 15 rabbits which were killed by bloodletting af

death, the mean lung weight was 7.71 ±2.80 g per kg body weight. In 30 bled rabbits with anuria for at least 3 days, the mean lung weight measured immediately after death was 4.19 ±0.4 g per kg body weight, and in 39 rabbits which died spontaneously in uraemia and were examined within 1 / hours of death, it was 4.98 ±0.64 g per kg body weight. The difference between the first and the last two series is significant. Hence large amounts of fluid (blood or serum) must be transported to the lungs during the first 24 hours after death. These findings thus agree with those obtained by Durlacher et al. (73) under different experimental conditions.

The water content in the lungs. In 21 rabbits which died spontaneously in uraemia and were examined within 24 hours of death, the mean content of water in the lungs was 82.6 ±2.5 %. For comparison may be used 39 rabbits (see section 2 above) which died spontaneously in uraemia and were examined within 1 hours of death, with a mean of 80.9 ±1.5 % and 30 bled severely uraemic rabbits with anuria for at least 3 days in which the mean value was 81.2 ±1.2 %. The difference is significant. The increase of the water content in the lungs after death, which in comparison with the increase of the lung weight is relatively moderate and the protein-rich alveolar oedema (see below) suggest that after death blood-cells and proteins are transported to the lungs, whereas the water conveyed to the lungs is less than in pulmonary oedema in vivo.

Microscopical examination. In 13 out of 14 rabbits which died spontaneously and were examined within 24 hours of death, profuse to moderate alveolar oedema was present even in those whose lungs weighed only 6—7 g per kg body weight. The alveoli contained large amounts of precipitated protein. hyperaemia in the alveolar walls was typical, whilst red blood cells and alveolar phagocytes were rarely seen in the alveoli.

The reason why *postmortal pulmonary oedema* in these experiments occurred to such varying extent is not clear. The position of the body is an important factor in that the lowest parts of the lungs were always most oedematous. Overhydrated rabbits which were examined a long time after death had high lung weights. Other factors, for instance the rate at which the blood coagulated after death, may possibly have played a part. In this study only the significance of uraemia has been considered. Durlacher's et al. experiments have shown that the manner of death is a factor of importance.

The following experiments were made in order to throw further light on the problem.

10 normal and 8 severely uraemic rabbits were killed by intravenous injection of 10 ml of air. X-ray (6 rabbits) showed postmortally no abnormalities in the lungs but cardiac enlargement. Autopsy was performed on all the 18 animals immediately after death. None had frothy fluid in the airways. In 2 normal animals there was gross evidence of marked hyperaemia. The water content of the lungs was 81.4 (80.3—83.5) %. The lung weight was f the normal rabbits 5.38 (4.29—8.00) and

death it was 5 15 (4 01—6 76) g and in 11 examined within 90 minutes of death 5 19 (4 12—6.55) g *per kg body* weight Table 9 shows data for the animals with the highest lung weights. Table 7 In all the 39 rabbits the mean lung weight was 4 98 ± 0 64 g per kg body weight or expressed with 2 ½ sigma *3.38* ± *6 58* g per kg body weight. These figures have been chosen as "normal values" for spontaneously dead rabbits in these studies.

(*b*) The *water content of the lungs* In the 12 rabbits in which the measurements could be started within 10 minutes of death the mean water content of the lungs was 81 0 (80.3—82 7) % In *rabbits examined within 11—80* minutes of death the value was 80 7 (78 2—84 6) % and in 11 rabbits examined within 90 minutes of death it was 81 0 (79 0—83 2) % In 39 rab*bits which died spontaneously in their* uraemia (Table 7) the mean water content of the lungs was 80 9 ± 1.5 % or expressed with 2 ½ sigma, *77 1—84 7* %

Microscopical examination In some animals small areas of atelectasis were seen, but in most cases the findings did not differ from those in the bled animals. There were some exceptions, *namely rabbits with a high lung weight* (Table 9) which exhibited hyperaemia and, in 3 cases, oedema in a few alveoli as well.

Examination 24 hours after death of animals with rigor mortis

21 rabbits *which died spontaneously* in uraemia 16 normal rabbits, and 15 rabbits with severe uraemia which were killed by *intravenous injection of 10* ml of air were examined after death *At observation of the first* mentioned group rigor mortis was found to begin in the neck about 1½ hours after death

Gross observation The lungs of the rabbits were either bright red and *light, or heavy and dark red to bluish* purple with diverse mottled markings. In those of the latter appearance the picture resembled that seen after death in rabbits with pulmonary oedema *The lung markings were distinguish* able from pulmonary oedema in that they were more symmetrical and extended peripherally and into all the lobes, but the lung surfaces were in *these cases so markedly changed that* it was impossible to diagnose any *slight* or moderately severe changes that could have been present in vivo Thus, in rabbits which had been dead *for 24 hours, pulmonary oedema could* not be diagnosed but in many cases its presence could be excluded.

Frothy fluid in the air ways In 4 out of 21 rabbits (19 %) which had died spontaneously in their uraemia and in 6 out of 31 (19 %) which had been killed by *intravenous injection of* air frothy fluid was demonstrated in the air ways On the other hand, in 29 animals which had died in uraemia and 20 which had been killed by injection of air no frothy fluid was found at examination while their bodies were still warm and soft *This would sug*gest that frothy fluid in the air ways can form post mortem

Lung weight In 21 rabbits which died spontaneously in their uraemia *and were examined* on the day after

Symptoms and pulmonary oedema at overhydration

The experiments comprise the following series I. Increase of the blood volume 1) blood transfusions, 2) infusion of dextran solution II. Increase of the extracellular fluid 1) infusion of saline 2) infusion of mannitol solution III. Increase of all the fluid spaces 1) infusion of glucose solution, 2) infusion of urea solution.

Increase of blood volume

Blood transfusion

The first known blood transfusion was given to Pope Innocentius VIII in 1492. The three donors died and the Pope became severely ill. Lower (1665) performed the first successful transfusion in animals. It was only after Landsteiner's discovery of the blood-groups in 1901 that blood transfusion was widely used therapeutically.

Pulmonary oedema after blood transfusion in man has been described by several authors (57, 88, 67, 103, 122, 141, 191, 220), notably in uncompensated heart failure, chronic nephritis, chronic anaemia, massive haemorrhage, and severe infection.

Sharpey-Shafer & Wallace (1942) infused into normal subject up to 2000 ml of blood, serum, or isotonic saline for about ½ hour. The venous pressure rose to, at most 11 cm H_2O but fell rapidly except after transfusion of blood. Chest X-ray showed distinct lung densities at the hili and slight cardiac enlargement. Vital capacity decreased. Electrocardiography showed abnormalities indicating slight right heart stress.

Müller (1874) gave infusions of blood to dogs intravenously up to 17 % of the body-weight for 1—3 hours. No pulmonary oedema was noted, but hyperaemia, numerous small haemorrhages, and exudate in the pleural cavities and the abdominal cavity occurred. In experiments with blood transfusions in dogs carried out by Cohnheim & Lichtheim (1877) pulmonary oedema failed to appear in most cases, but numerous punctiform haemorrhages in the lungs arose. Krumbhaar & Chanutin (1922) increased the total red-cell volume to three times the normal by daily blood transfusions the plasma volume, on the other hand, decreased pulmonary oedema did not develop, not even in a dog which lived for 40 days with twice the normal blood volume. Gibbon & Gibbon (1942) reduced the lung tissue in cats by lobectomy these animals developed pulmonary oedema after one transfusion of a blood volume that was well tolerated by normal animals. In the presence of pulmonary oedema the pressure in the pulmonary artery was high but that in the left atrium normal. Luisada & Sarnoff (1946) administered large amounts of isotonic saline solution, Tyrode solution, bovin albumin-salt solution, and blood separately in the carotid arteries. The total amount of injected fluid was 1—4 times the animal's estimated blood volume. The infusion was performed within half

for the uraemic ones 4 15 (3 66—5 47) g per kg body weight.

16 normal rabbits and 15 rabbits with severe uraemia were killed by injection of 10 ml of air intravenously and examined within 24 hours of death In the normal rabbits the mean lung weight was 6.29 ± 1 06 g per kg body weight and in the uraemic rabbits it was 7 00 ± 1 51 g per kg body weight The difference is not significant The mean water content of the lungs was for the normal animals 81 8 ± 1 4 % and for the uraemic animals 83 7 ± 1 9 % The difference is not significant.

Examination by this technique did not show that the tendency to pulmonary oedema after death was significantly higher in uraemic than in non uraemic rabbits.

Uraemia and pulmonary oedema

In rabbits that died in uraemia, pulmonary oedema was not present at autopsy immediately after death The tendency to pulmonary oedema appearing after death was not significantly higher in uraemic than in non uraemic rabbits.

Survey See page 145

CHAPTER 6

Symptoms and pulmonary oedema at overhydration

The experiments comprise the follow ing series. I. Increase of the blood volume: 1) blood transfusions, 2) infusion of dextran solution. II. Increase of the extracellular fluid: 1) infusion of saline 2) infusion of mannitol solution. III. Increase of all the fluid spaces: 1) infusion of glucose solution, 2) infusion of urea solution.

Increase of blood volume

Blood transfusion

The first known blood transfusion was given to Pope Innocentiu VIII in 1492. The three don rs died and the Pope became severel ill. Lower (1665) performed the first successful transfusion in animals. It was only after Landsteiner discovery of the blood-groups in 1901 that blood transfusion was widely used therapeuti cally.

Pulmonary oedema fter blood transfusion in man has been described by several uthors (57 58, 67 103 132, 141 191 228), notabl in uncompensated heart fail hronic nephritis, chronic anaemia, massiv haemorrhage and sever infections.

Sharpey Shafer & W llace (1912) infused into normal subject up to 2000 ml of blood, serum, or isotonic saline for about ½ hour. The enous pressure rose to, t most, 11 cm H_2O but fell rapidly ex ept fter transfusion of blood. Chest X ray howed distinct lung densities at the hili and slight cardiac enlargement. Vital capacity decreased. Electrocardiography showed abnormalities indicating slight right heart stress.

Müller (1874) gave infusions of blood to dogs intravenously up to 17 % of the body weight for 1—2 hours. N pul monary oedema was noted, but hyper aemia, numerous small haemorrhages, and exudate in the pleural cavities and the abdominal ca ity occurred. In experiments with blood transfusions in dogs carried out by Cohnheim & Lichtheim (1877) pul monary oedema failed t ppear in most cases, but numerous punctif rm haemor rhages in the lungs arose. Krumbhaar & Channtin (1922) increased the total red-cell volume t three times the normal by daily blood transfusions the plasma vol ume on the other hand decreased pulmonary oedema did not develop, not even *in a dog which lived for 40 days with* twice the ormal blood olume. Gibbon & Gibbon (1942) red ced the lung tissue in cats by lobectomy these animals devel ped pulmonary oedema after one transfusion of a blood olume that was well tol erated by normal animal In the presence of pulmonary oedema the pressure in the pulmonary artery was high but that in the left trium normal. Lubeda & Sarnoff (1948) administered large amounts f isotonic saline solution, Tyrode solution, bovine albumin-salt sol tion, and blood separately in the carotid arteries. The total amount of injected fluid was 1—3 times the animal's estimated blood volum The infusion w performed within half

an hour The incidence of pulmonary oedema was highest after infusion of blood, lower after albumin salt solution, and lowest after saline solution. Huckabee et al. (1950) induced hypervolaemia in 10 dogs by intravenous infusion of blood or albumin plus fluid to decompensation In some cases more than 20 % of the body weight was given for ½ to 2 hours. Pul monary oedema was produced more read ily by quick than by slow infusion more readily with 10 % than with 5 % albumin solution, and the incidence was lowest after infusion of whole blood Young et al. (1955) gave dogs blood transfusions to almost double the original blood volume at a rate of 1 ml per minute and kg pul monary oedema did not develop the pres sure in the right atrium did not rise.

Aikawa (1950) found that the blood vol ume in the rabbit was 7 0 % of the body weight Jung (1958) quotes a value of 5 % and Drenckhahn (1958) 7 7 %

Own investigations

In 21 uraemic rabbits, blood in amounts equalling 12 % of the ani mals body weights was transfused during 1 ½ hours. In 10 of the rabbits the ureters were tied 48 hours before the transfusion and in 11 the operation was made immediately before the transfusion The animals were killed by bloodletting about 4 ½ hours after the completion of the transfusion.

The transfused volume and the time of bloodletting were the same as in the ex periments with dextran described under heading 2

Blood for transfusion was obtained by cardiac puncture from 5 donors. The blood was mixed and 10 mg of heparin were added. Blood samples for cross tests of the donors blood-corpuscles and the recipient s serum were drawn from the ears (donors) and the heart (recipient) Cross matching at 37 C was done at least 1 week, on an average 15 days, before the transfusion. Incompatibility was found in 7 % of the tests.

Performed at the Blood Bank of the U i er sity Hospital, Lund, Sweden, (Head Bengt Löw)

Symptoms in vivo

Appearance and behaviour In most cases exophthalmos developed after the transfusion, but no or only slight, dyspnoea was noted. The animals were slow in reacting moved very little, and trembled slightly but their muscular strength was good. The degree of uraemia seemed to have little influence on the symptoms. Convulsions and ny stagmus were not noted

The respiratory rate fell during the transfusion from 170 (88—200) to 110 (80—208) per minute. Immediately be fore the bloodletting it was 125 (72—180) per minute The more highly uraemic animals had a lower respira tory rate

The heart rate was 239 (1?0—308) and 239 (91—52?) beats per minute before and after the transfusion, re spectively Immediately before the bloodletting the rate was ?33 (120—316) beats per minute The difference was small between slightly and mode rately uraemic animals. The highest value (522) was recorded for a rabbit with ventricular fibrillation

Electrocardiographic abnormalities occurred in 7 out of 11 slightly uraemic animals and persisted or were accen tuated in 6 out of 10 moderately uraemic animals during the transfu

sion. At the completion of transfusion 8 out of 21 rabbits had normal electrocardiograms, 4 had flat T waves, 3 had negative T waves, and 6 had severe disturbances, such as arrhythmia, deformation of the QRS-complex, ventricular fibrillation, etc. At the bloodletting 4 1/2 hours later the electrocardiograms were in 9 cases as before, the changes had deteriorated in 5, and improved in 7 The 3 rabbits with evidence of pulmonary oedema had after the transfusion a normal electrocardiogram, flat T waves, and ventricular fibrillation, respectively In the last mentioned case improvement was noted *the other two deteriorated up* to the bloodletting and thereafter the T waves were flat and negative, respectively

Marked pulmonary changes appeared in the radiogram (3 of 21 films were spoilt) after the transfusion in 1 rabbit slight changes were seen in 6, whereas the picture was still normal in 11 *Immediately before the blood-*letting (Table 10) 2 out of 21 rabbits had marked radiographic changes (both had at autopsy heavy pleural haemorrhage 75 ml and 91 ml, respectively) nd 4 had slight changes. At autopsy after bloodletting pulmonary oedema evidenced by three criteria wa noted in 1 of the last named 4 animals, whilst no pulmonary changes were found in the other 3 Severe hyperaemia could possibly have produced some of the above-mentioned radiographic pulmonary changes.

The radiographic heart size was before the transfusion slightly increased in 1 case After the transfusion it was normal in 7 slightly increased in 8, moderately increased in 2, and non-assessable in 4 cases. Immediately before the bloodletting (Table 10) the heart was of normal size in 5 slightly enlarged in 8, *moderately* enlarged in 5 and non-assessable in 3 rabbits. In no animal did the heart size decrease during the period after the transfusion. No difference could be established between slightly and moderately uraemic rabbits.

Postmortem findings

On cutting the carotid artery at the bloodletting, about twice as much *blood as the usual amount*, 64 (30—110) ml, spurted from the artery under high pressure the average being 127 (80—180) ml. In 2 rabbits large *amounts of bloody fluid were found* in the pleural cavities. Eight rabbits had highly red-coloured and 4 lightly red-coloured ascitic fluid. At autopsy minor subcutaneous *haemorrhages* were seen, but there was no melaena On the lung surfaces were seen in 4 cases a few and in 3 cases numerous red dots, in 4 cases red spots, and in 1 case confluent ill-defined red spots in the hilar region. In 1 rabbit the lung surface was red pink spotted. In all the cases the fluid that could be squeezed out of the lung was sparse.

Frothy fluid in the air ways was noted in only 2 cases.

The lung weight exceeded the normal in 4 cases the highest lung weight was 6.46 g per kg body weight (Table 10).

The water content of the lung was higher than normal in 1 case.

most to 2.3 times the original value, and the pressure in the right atrium to 120 mm saline. Autopsy showed no evidence of pulmonary oedema. Marx et al (1960) infused 6 % dextran in isotonic saline solution intravenously in dogs, 1 ml per minute and kg for 1 hour. Pulmonary oedema did not develop.

Goldenberg et al. (1947) showed that haematocrit and total protein in serum decreased markedly after infusion of dextran in rabbits. The concentration of dextran in blood fell to about 25 % of the original value after 24 hours. As the excretion was insignificant (6 %) and no accumulation in the tissues was noted it was considered probable that dextran is rapidly metabolized in rabbits. Howard et al. (1961) observed that dextran was eliminated from plasma almost equally quickly in oliguric as in non-oliguric patients: about 15 % was present in the circulating blood after 24 hours. Distribution of dextran to the extracellular space could only partly explain the continued marked decrease of the dextran concentration, and, consequently dextran must be deposited or metabolized. Wasserman et al. (1955) found that the rate at which dextran disappeared from the circulating blood in dogs depended upon its molecular weight. The concentration of dextran in blood and lymph was the same only for the smallest molecules (molecular weight about 10 000). The heavier molecules occurred in higher concentration in blood than in lymph. At infusion of large amounts, there was, however very little difference in the dextran concentration between blood and lymph even for dextran with very high molecular weights (about 300,000). Space for dextran with a molecular weight of 60,000 and 400 000 respectively was the same. At a molecular weight of 10,000—35 000 dextran was distributed in a space equalling that of inulin.

The dextran solution was kindly placed at our disposal by AB Pharmacia Uppsala Sweden.

At administration of large amounts of dextran, as in the experiments described in this chapter, the animals are made anaemic. A short review of the part played by anaemia in causing pulmonary oedema would therefore be appropriate. The problem has been studied by for instance, Eaton (1947) who was able to produce pulmonary oedema, haemorrhages, and hyperaemia in dogs by heavy loss of blood. The pressure in the systemic arteries fell, but that in the pulmonary artery rose. The pulmonary oedema was aggravated by infusion of saline solutions. Földi et al (1948) showed that there was a correlation between haemoglobin content and capillary filtration. They considered that oedema in anaemic patients would be due to a pathological increase of permeability in the capillary walls. Fowler et al (1958) found that in dogs which were hypervolaemic after dextran infusion cardiac output increased only if the animals were anaemic as well. Tybjerg Hansen (1962) formed the hypothesis that the red cells would be important vehicles of fluid and take on fluid at their passage through the capillaries.

Own investigations

The experimental series consists of 41 rabbits. 6 % dextran solution in 0.9 % sodium-chloride solution to which 0.24 g of calcium chloride and 0.42 g of potassium chloride per litre had been added, was infused intravenously in uraemic rabbits, in an amount equalling 12 % of the body weight for 1 ½ hours (1.3 ml per minute and kg). The dose of 12 % was chosen since preliminary experiments had shown that almost all the rabbits developed pulmonary oedema if an amount as large as 15 % of the body weight was given. 11 rabbits received the infusion within 1 hour, 10 within 24 hours, 10 within

48 hours, and 10 within 72 hours of the ureteral ligation. The animals that did not die spontaneously were killed by bloodletting 4—5 hours after the infusion. This interval of time was chosen because in preliminary experiments it had been found that dyspnoeic animals began to improve a few hours after the infusion, despite increasing uraemia, and that the dyspnoea lessened gradually or disappeared.

It will be seen from Table 11 that 27 out of 41 animals lived until the planned bloodletting whereas 14 died spontaneously after the infusion. Among the latter 14 animals, 2 had been anuric for only a few hours, 4 for 24 hours, 2 for 48 hours, and 6 for 72 hours. In these animals, autopsy was performed within an average period of 19 (5—45) minutes of death.

Symptoms in vivo

Appearance and behaviour The animals became drowsy after the infusion they did not move but sat cowering and did not react to light prodding. Just before being caught, however they managed to hop about a little, with the exception of the severely uraemic ones, some of which were too weak to move The eyelid-closure reflex was weak Spasmodic twitchings arose in some cases. Exophthalmus occurred in many animals during the infusion.

The respiratory rate fell during the infusion from an average of 122 (88—180) to 107 (52—190) per minute With their noses in the air the animals breathed so quietly that the alae nasi hardly moved. In 8 animals with three criteria of pulmonary oedema and anuria for at least 48 hours, the respiratory rate immediately before blood letting averaged 128 (100—160) per minute, whereas in 17 without three criteria of pulmonary oedema and, in most cases, slight uraemia the average rate was 84 (48—124) per minute. With one exception, all the animals with three criteria of pulmonary oedema had dyspnoea. Slight dyspnoea was also noted in 6 animals and severe dyspnoea in 1 animal without three criteria of pulmonary oedema.

E.C.G and heart rate The heart rate before and after the dextran infusion averaged 217 (147—300) and 256 (129—340) beats per minute respectively In 10 animals with three criteria of pulmonary oedema, mostly of slight degree, the average heart rate immediately before the bloodletting was 255 (174—294) beats per minute in 16 rabbits without three criteria of lung oedema it was 270 (200—294) beats per minute.

The electrocardiograms were before the infusion normal in 31 rabbits in 15 of them pulmonary oedema developed later T wave flattening was seen in 5 out of 41 rabbits before the infusion in 4 of them pulmonary oedema arose later Five of the 41 rabbits had before the infusion negative T waves or deformed QRS-complexes in all of them pulmonary oedema developed later In comparison with electrocardiograms recorded immediately before the infusion, no or slight changes had occurred immediately after the infusion in 27 animals, deterioration

Table 11 *Results after infusion of dextran solution amounting to the equivalent of 12 % of the body-weight for 1 ½ hours in anuric rabbits whose ureters had been tied 0—2 24 48 and 72 hours respectively before the infusion L=at least three criteria of lung oedema S=spontaneous death*

		Lung weight g/kg body weight	Water content in lung	Pulmonary oedema by gross observation	Frothy fluid in the air-way	Alveolar oedema	X ray Lung changes	X ray Cardiac enlargement	Pleural fluid ml	Ascites ml	N P N mg per 100 ml	Post infusion survival hours	
I Dextran infusion 0—2 hours after ligation of ureters	L	11.32	87 1	+++	++	0	++	+	4	69	08	1	S
	L	7.02	83.8	++	+	0	+	+	1	62	66	4	
		6.78	82.4	++	0	0	0	0	0	0	00	4	
		4 73	83.8	0	0	0	+	++	4	85	30	5	
		4.08	82.7	0	0	—	0	0	0	44	01	5	
		4.86	81 7	0	0	—	0	++	0	15	55	4	
		4 35	82.8	0	0	0	0	0	0	2	84	4	
		4.27	81.0	0	0	—	0	0	0	04	60	5	
		1.37	81 7	0	0	0	0	0	0	2	02	3	S
		3 88	81.0	0	0	—	0	0	0	13	43	4	
		3.60	83.3	0	0	—	0	0	12	15	06	4	
Mean		5.49	83.0						2	34	70		
II. Dextran infusion 24 hours after ligation of ureters	L	11 75	87 7	+++	+++	0	+++	++	2	140	102	0.3	S
	L	10 46	83.0	++	+++	0	+++	+	3	226	118	4	S
	L	8.45	81 0	+++	++	0	+	++	3	212	114	5	
	L	7.80	81.9	+++	+++	0	+	++	3	210	93	0.3	S
	L	7 70	82.5	+	+	+	++	++	4	180	103	5	
	I	6.72	84.3	++	0	0	+	++	1	130	100	1	S
		5 41	82.5	0	0	0	+++	++	7	108	120	4	
		5 17	82.2	0	0	0	+	++	1	108	150	5	
		4.36	83.0	0	0	0	0	+	1	155	115	5	
		4 12	82.8	0	0	0	0	++	2	156	111	1	
Mean		7 70	83 8						3	172	115		

III Dextran infusion 48 hours after ligation of ureters (continued)	11.10	87	+ +	++	●	—	—	0	101	—	0.1 ¥
	11.83	90.0	+++	++	0	+++	+	12	77	157	0.2 ¥
	8.2	89.1	++	+	●	+	+	2	231	706	5
	7.31	87.7	++	+	0	+	+	0	170	136	8
	[illegible]	80.5	+++	+	0	++	+	2	136	270	4
	6.61	82.4	++	●	+	++	+	12	103	393	1
	5.35	67.7	+	0	0	+	++		280	311	4
	5.37	88.1	+	0	●	+	+	●	181	133	5
	5.01	83.●	+	0	0	●	+	0	105	703	4
	3.75	81.●	0	0	—	+	+	1	53	313	1
Mean	81	81.0						4	149	70	
IV Dextran infusion 72 hours after ligation of ureters	13.33	88.5	+++	++	+	+++	++	●	75	211	0.1 ¥
	9.11	87.2	+++	++	+	+++	++	●	167	318	0.1 ¥
	9.01	81.4	++	+	+	++	++	2	233	—	4 ¥
	8.17	85.1	+++	++	+	—	—	4	75	378	0 ¥
	6.90	83.3	+	+	++	0	++	7	182	233	4
	6.76	83.4	+++	++	++	++	+	10	125	200	0 ¥
	6.78	81.9	++	+	+	—	—	13	135	303	2 ¥
	6.11	81.5	0	+	0	+	+	1	80	197	4
	5.51	80.6	+	●	+	0	++	4	168	276	6
	4.17	81.2	0	0	—	0	0	2	170	211	6
Mean	07	81.1						5	136	218	

in 9 and *improvement in 1* (4 rabbits died during or shortly after the infusion) *Up to the time of bloodletting* the electrocardiograms showed further changes in 9 cases After the infusion electrocardiographic abnormalities were seen in 1 out of 11 rabbits with uraemia for a few hours, in 5 out of 10 with uraemia for 24 hours, in 7 out of 9 with uraemia for 48 hours, and in 3 out of 7 with uraemia for 72 hours At the time of bloodletting 6 rabbits had negative T waves or deformation of the QRS-complex one of them was found to have three criteria of pulmonary oedema

The size of the heart was before the infusion *normal in 38 out of 41 animals* and increased in 3 all the latter *three developed pulmonary oedema* during or after the infusion *After the infusion the heart size was increased* in 31 out of 39 animals (2 died during the infusion) among which pulmonary oedema evidenced by at least three criteria arose in 19 The heart was of normal size in 8 animals one of which developed slight lung oedema It will be seen from Table 11 that immediately before the bloodletting 10 animals had moderately (++) increased heart size 5 of these had three criteria of pulmonary oedema. Out of 15 animals with three criteria of pulmonary oedema which were examined before death all had cardiac enlargement Among 14 animals which died spontaneously 5 were X rayed before the respiratory standstill and had enlarged hearts, 6 were X rayed within 5 minutes of the respiratory standstill and of these 2 had enlarged hearts and 4 normal sized hearts, and 3 were not X rayed (more than 5 minutes had elapsed after the respiratory standstill)

X ray of the lungs was normal in all the 41 animals before the infusion. In 21 pulmonary changes were noted at the end of the infusion 5 of those which died spontaneously before the planned bloodletting showed changes coded as +++ 2 which died spontaneously and 4 bled animals had ++" and 10 bled animals had + In 2 of the last mentioned animals the degree increased to ++" before the bloodletting

It will be seen from Table 11 that immediately before the bloodletting or *in the agonal state 23 animals (56 %)* had pulmonary changes and that X *ray showed no abnormalities in 15* (37 %) 3 (7 %) were not examined *as more than 5 minutes had elapsed* after the respiratory standstill Five out of 23 animals with radiographic pulmonary changes had not a sufficient number of other criteria for the diagnosis of pulmonary oedema Three of these 5 had large haemorrhages whereas in 2 no changes were seen in the lungs at autopsy after bloodletting (hyperaemia?) Two rabbits with at least 3 criteria of pulmonary oedema had normal lungs on X ray immediately before the bloodletting both had numerous small haemorrhages macroscopically alveolar oedema microscopically and their lung weights were slightly above the upper limit of normal range 1 rabbit had frothy fluid in the bronchi

Postmortem findings

Gross observation at autopsy showed normal lung surface in 1 rabbit and haemorrhages in 40 in the form of a few to numerous red dots or spots within a limited area or over the entire surface of the lung The most severe changes were seen in animals with high-degree uremia. In 20 rabbits the lung surface had the spotted or mottled appearance typical of pulmonary oedema ("+++ or ++") while in 6 the pulmonary changes were assessed as mild (+) Two animals with slight macroscopical pulmonary changes, numerous small red and white dots or red spots, had not a sufficient number of other criteria of pulmonary oedema. In 1 rabbit the lung surface was pink and smooth but pulmonary oedema was present according to four other criteria.

Frothy fluid in the air ways was found in 20 rabbits In 3 of these frothy fluid flowed from the nose and mouth Four animals with slight pulmonary oedema by at least three criteria had no frothy fluid in the air ways.

The lung weights were in 24 out of 41 increased (Table 11) the increase was xplained by pulmonary oedema, haemorrhage and/or in spontaneously dead animals, by hyperaemia. One animal had light pulmonary oedema by three criteria (X ray and gross findings, and water content) but normal lung weight. In 1 the lung weight was hov the upper limit of normal range or 6.78 g per kg body weight and numerous diffuse red spots were seen but microscopical examination showed haemorrhages without alveolar oedema there was no frothy fluid, X ray showed no abnormalities, and the water content was normal—haemorrhage.

The water content of the lungs was increased in 15 out of 41 animals (Table 11) 11 rabbits with pulmonary oedema evidenced by at least three criteria had normal water contents. In 2 rabbits with slightly increased water contents, a sufficient number of criteria for the diagnosis were not satisfied.

In spite of high lung weights, profuse amounts of fluid macroscopically (often bloodstained, however) on the section surface, and microscopically very protein-poor oedematous fluid, the water content seldom reached very high values. This suggests that haemorrhages and hyperaemia were responsible for a considerable part of the increase in lung weight.

Microscopical examination showed alveolar oedema in 10 cases. In 14 cases of pulmonary oedema evidenced by at least three criteria, there was no alveolar oedema. The absence of visible alveolar oedema may be explained by protein poor oedema fluid with low stainability which has earlier been described by for instance, Visscher et al., or the explanation may be that alveolar oedema was not present Arguments against the latter assumption are that *oedema fluid flowed* profusely from the section surfaces of the lungs and that no alveolar oedema was seen in 9 animals which had pulmonary oedema by all other criteria. The bronchioles showed no changes. In the

arteries there was no periarterial—interstitial oedema.

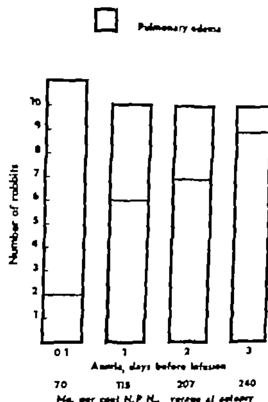


Diagram 7 Occurrence of pulmonary edema afte infusion of dextran solution in amounts equalling 12 % of the body weight for 1½ hours in rabbits which had been anuric for 0.1 2, 2, and 3 days, respectively before the infusion.

Criteria of pulmonary oedema

The six criteria of pulmonary oedema used here agree less well in this than in the other series The diagnosis of pulmonary oedema was established in 24 cases and in 8 % it was supported by all the six criteria In 54 % by five, in 25 % by four and in 13 % by three criteria (Radiographs were *missing in* 3 cases) Among these 24 cases alveolar oedema could not be demonstrated in 58 % and the water content was *normal in 37 % However only 2 out* of the 24 animals with pulmonary oedema by at least three criteria had neither alveolar oedema nor a water *content above the upper limit of* normal range.

In 17 cases the number of positive criteria was less than three, and pulmonary oedema was therefore not considered to be present Two of these had no positive criteria, 12 had one criterion, and 3 had two criteria of pulmonary oedema.

It will be seen from Diagram 18 and Table 11 that pulmonary oedema following dextran infusion in uraemic rabbits is characterized by marked macroscopical changes, among which haemorrhage predominates, high lung weights in most cases clear X ray evidence of *pulmonary changes and* frothy fluid in the air ways but also by a moderately or slightly increased water content and no or insignificant *alveolar oedema*

The great diagnostic difficulties in this series were attached to the differ*entiation between pulmonary oedema* and pulmonary haemorrhage. Bleeding of varying degrees was noted in 98 % of all the rabbits, and all those *that had pulmonary oedema* by at least three criteria had also haemorrhages into the lung parenchyma. The absence of alveolar oedema may pos*sibly be explained* by a low protein content in the oedema fluid the low water content may be attributable to profuse amounts of red cells and possibly dextran in the lungs This series shows that several methods must be used in evaluation of the oedema state.

so as to ensure a reasonably accurate calculation of the frequency of pulmonary oedema. Yet, despite the use of six methods of examination, the diagnosis of pulmonary oedema is uncertain in the borderline cases.

Pulmonary oedema and uraemia

It will be seen from Table 11 and Diagram 7 that, according to the definition used here, pulmonary oedema arose after dextran infusion in 2 out of 11 rabbits with anuria for less than 10 hours, in 6 out of 10 with anuria for *24 hours, in 7 out of 10 with anuria* for 48 hours, and in 9 out of 10 with anuria for 72 hours. The incidence of pulmonary oedema rose significantly with increasing uraemia. After infusion of dextran in uraemic rabbits the *incidence* of pulmonary oedema was higher than after transfusion of the same amount of blood (0 05 $<$ p $<$ 0 10)

Increase of the extracellular fluid

Infusion of saline

Reviews of the literature on pulmonary oedema have been presented by several authors (e.g. 117 168, 46, 247)

Ma y in estigation ha been made into the effect of infusion of saline solution in man. Pulmonary oedema i man after infusion ha been described (6—21 *82 182, 184, 196* and others) Galligan et l. (1938) usi g infusion of 800—1600 ml of saline found that the haematocrit and serum protein values f ll and *that the* blood olume increased lightly With hypertonic solution the *increase as* somewhat greater than with isotonic solutions. The said disorders la ted f 2 hours t most. Doyle et l. (1951) fter saline infusion, observed increased blood volume and raised pressure in the lung capillaries but no disproportionally raised pressure in the pulmonary circulation as compared with the systemic circulation.

The effect of infusion of saline solution in animals with good renal function has been studied by many authors. Cohnheim & Lichtheim (1877) produced pulmonary oedema in rabbits, dogs, sheep, goats, and *horses by rapid infusion of large amounts* of 0.6 % sodium-chloride solution intravenously Pulmonary oedema, as seen at gross examination, arose in only a small numbe of the animals, however: it was mildest in rabbits, although the infusions were continued until the animals died. Rabbits tolerated an amount of up to 46 % of the body weight infused for 2 hours (about 4 ml per minut and kg) Dastre & Loye (1888) found that intravenous infu ion of t least 3 ml per minute and kg was required t produce pulmonary oedema in rabbits, the total amount *being* less important. Knoll (1895) also infused 0.6 % saline at a rate of 3 ml pe minute and kg body-weight and found that a f w rabbits did not die until an amount highe than the body weight had been given. Tracheotomized animals tolerated *larger* mounts than did non tracheotomized animals, which was thought t be explained by the fact that profuse nasal discharge made breathing difficult in non-tracheotomized animals. Warthen (1935) wa able to produce pulmonary oedema in dogs but nly by intravenous *injection for* 24 minutes, t a rat of 10 ml per minut and kg. Lahada & Sarnoff (1948) *found* that pulmonary oedema was more readil produced by infusion into the *carotid ar*tery than by infusion int the femoral artery or a ein.

Cohnheim & Lichtheim (1877) noted *that acute infusion* caused an insignificant rise in blood-pressure and a marked fall of *the protein* content in the blood-serum. The *flow of lymph from* the thoracic duct *increased 23-fold*. The increase originated almost entirely from the abdominal or

arteries there was no periarterial—in terstitial oedema

Criteria of pulmonary oedema

The six criteria of pulmonary oedema used here agree less well in this than in the other series The diagnosis of pulmonary oedema was established in 24 cases and in 8 % it was supported by all the six criteria in 54 % by five in 25 % by four and in 13 % by three criteria. (Radiographs were missing in 3 cases) Among these 24 cases alveolar oedema could not be demonstrated in 58 % and the water content was normal in 37 % However only 2 out of the 24 animals with pulmonary oedema by at least three criteria had neither alveolar oedema nor a water content above the upper limit of normal range

In 17 cases the number of positive criteria was less than three, and pulmonary oedema was therefore not considered to be present Two of these had no positive criteria, 12 had one criterion, and 3 had two criteria of pulmonary oedema.

It will be seen from Diagram 18 and Table 11 that pulmonary oedema following dextran infusion in uraemic rabbits is characterized by marked macroscopical changes, among which haemorrhage predominates, high lung weights in most cases clear X ray evidence of pulmonary changes, and frothy fluid in the air ways but also by a moderately or slightly increased water content and no or insignificant alveolar oedema.

The great diagnostic difficulties in this series were attached to the differentiation between pulmonary oedema and pulmonary haemorrhage Bleeding of varying degrees was noted in 98 % of all the rabbits, and all those that had pulmonary oedema by at least three criteria had also haemorrhages into the lung parenchyma The absence of alveolar oedema may possibly be explained by a low protein content in the oedema fluid the low water content may be attributable to profuse amounts of red cells and possibly dextran in the lungs. This series shows that several methods must be used in evaluation of the oedema state

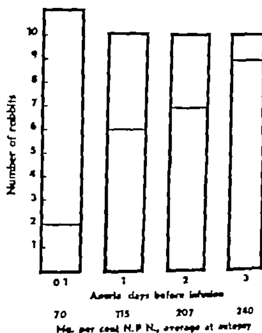


Diagram 7 Occurrence of pulmonary edema after infusion of dextran solution in amounts equalling 12 % of the body weight for 1 ½ hours in rabbits which had been anuric for 0 1 2, 2, and 3 days, respectively before the inf sion.

so as to ensure a reasonably accurate calculation of the frequency of pulmonary oedema. Yet, despite the use of six methods of examination, the diagnosis of pulmonary oedema is uncertain in the borderline cases.

Pulmonary oedema and uraemia

It will be seen from Table 11 and Diagram 7 that, according to the definition used here, pulmonary oedema arose after dextran infusion in 2 out of 11 rabbits with anuria for less than 10 hours, in 6 out of 10 with anuria for 24 hours, in 7 out of 10 with anuria for 48 hours, and in 9 out of 10 with anuria for 72 hours. The incidence of pulmonary oedema rose significantly with increasing uraemia. After infusion of dextran in uraemic rabbits the incidence of pulmonary oedema was higher than after transfusion of the same amount of blood ($0.05 < p < 0.10$)

Increase of the extracellular fluid

Infusion of saline

Reviews of the literature on pulmonary oedema have been presented by several thors (e.g. 117, 168, 246, 247)

Ma y investigation have been made into the effect of infusion of saline solution in man. Pulmonary oedema in man after infusion has been described (6—21, 91, 103, 136, 194 and others). Gilligan t al. (1938) using infusion of 500—1500 ml of saline found that th haematocrit and serum-protein values fell and that the blood lum increased slightly. With hypertonic solutions the increase was somewhat greater than with isotonic solutions. The said disorders lasted for 2 hours t most. Doyle et al. (1951) after saline infusion, observed increased blood volume and raised pressure in the lung capillaries but no disproportionally raised pressure in the pulmonary circulation as compared with the systemic circulation.

The effect of infusion of saline solution in nimals with good renal function ha been studied by many authors. Coh helm & Lichtheim (1877) produced pulmonary oedema in rabbits, dogs, sheep, goats, and horses by rapid infusion of large amounts of 0.6 % sodium-chloride solution intravenously. Pulmonary oedema, as seen at gross examination, arose in only a small number of the a imals, however; it wa mildest in rabbits, lthough the infusions were continued until the animal died. Rabbits tolerated an amount of up to 46 % of the body weight infused for 2 hours (about 4 ml per minute and kg). *Dastre & Loye* (1888) found that intraven us infusion of at lea t 3 ml per minute and kg was required t produce pulmonary oedema in rabbits, the total amount being less important. Knoll (1893) also infused 0.6 % saline at rate of 3 ml per minute and kg body weight and found that a few rabbits did not die until an amount higher than the body-weight had been given. Tracheotomized animals tolerated larger amounts than did non tracheotomized animals, which was thought t be explained by the f ct that profuse nasal discharge made breathing difficult in non-tracheotomized animals. Warthen (1935) was able to produce pulmonary oedema in dogs but only by intravenous injection for 35 min tes, t a rate f 10 ml per minute and kg. Lafsada & Sarnoff (1946) *found* that pulmonary oedema was more readily produced by infusion int the carotid r tery tha by inf sion into the femoral artery or a vein.

Cohnheim & Lichtheim (1877) n ted that saline infu i n caused an insignificant rise in blood-pressure and marked fall of the protein content in the blood-serum. The flow of lymph from the thoracic duct increased 23-fold. The increase *originated* lmost entirely from the abdominal or

gans, the lymph flow from the extremities and the head being insignificantly increased. Autopsy showed large amounts of ascitic fluid but rarely pleural effusion. In most animals gross examination of the lungs showed only oedema in the hilar region. Weed & McKibben (1919) noted that the cerebrospinal pressure in cats changed very little during infusion of Ringer's solution. Lands et al. (1940) showed that the greater part of infused saline was retained in the extracellular space. Yeomans et al. (1943) found that the pressure in the pulmonary and the portal veins of dogs rose more markedly than the systemic pressure at infusion of large amounts of saline intravenously. In one animal the pressure in the pulmonary veins rose to 44 cm H_2O, the heart size increased, and the serum protein concentration fell to half during infusion for 30 minutes; physical signs of pulmonary oedema did not appear. In animals with physical signs of pulmonary oedema (rales heard over the lung fields) the pressure in the jugular vein was not higher than that in animals without pulmonary oedema. Luisada & Sarnoff (1946) in infusion experiments with saline in dogs, observed a rapidly transient rise of arterial and venous pressure and heart rate. Electrocardiographic examination showed flat or inverted T waves. Opdyke et al. (1948) found that infusion of blood or saline in dogs increased the pressure in the left atrium more than that in the right. Haddy et al. (1950) noted raised pressure in the pulmonary veins of dogs with pulmonary oedema, according to gross observation at autopsy, after infusion of large amounts of saline. Harrison & Liebow (1954) came to the conclusion that infusion in dogs produced pulmonary oedema in those animals which had the highest fluid retention, the largest blood volume, and the highest pulmonary vein pressure.

The following authors have studied the effect of fluid infusion under varying experimental conditions.

Kraus (1913) showed that intravenous infusion of saline gave rise to frothy pulmonary oedema in vagotomized rabbits (see the chapter on vagotomy).

Reichsman (1946) demonstrated that pulmonary oedema could be produced regularly in rats which were breathing against inspiratory resistance when they were given saline by rapid intravenous infusion.

Courtice & Korner (1952) produced pulmonary oedema, graded by lung weight, in hypoxic rabbits by infusion of Ringer-Locke's solution. Hypoxia (11 % O_2 in the inhaled air) or infusion 160 ml per kg used each by itself did not produce pulmonary oedema.

Wiggers (1939) introduced intravenously 15 litres of saline into a medium-sized dog during the course of 3 hours without signs of developing pulmonary oedema. If, however, pulmonary pressures were increased still further by means of epinephrine, oedema readily supervened. Korner (1953) produced pulmonary oedema, recorded macroscopically and by lung weight, in animals which received both noradrenaline and infusion of saline. When given separately neither the noradrenaline nor the infusion in the doses used gave rise to pulmonary oedema.

Daniel & Cate (1948) found that "wet lung", that is, findings of large amounts of fluid in the lungs at autopsy, occasionally occurred in dogs whose chests had been traumatized experimentally. If saline, about 200 ml per kg, was given in addition to the traumatization, "wet lung" was invariably produced.

Alwall et al. studied rabbits whose ureters had been tied bilaterally (see Chapter 7).

Alwall, Lindqvist et al. (1953) gave saline intravenously, 25 % of the body weight, for 1 ½ hours (2.8 ml per minute and kg) to rabbits immediately after bilateral ligation of their ureters. Within 48 hours slight radiographic pulmonary changes of "fluid lung" type (+) were seen in 60 % of the cases, slight gross changes as in pulmonary oedema in 0 % and increased

water content (80 % but no frothy fluid was found in the air ways, the lung-weight was normal, and no alveolar oedema was seen. No rabbit had three positive criteria of pulmonary oedema. On the other hand, profuse pleural effusion, a eraging 35 (16—41) ml, and atelectasis were noted.

Own investigations

The experimental series comprised 65 rabbits whose weights ranged between 2.20 and 2.49 kg At various times after ureteral ligation the animals were given isotonic salt solution intra venously 15 % of the body weight for 1 1/2 hours. The purpose was to find out whether different degrees of urae mia would play a part in causing pul monary oedema. This series forms the *basis for the experiments that will be* reported in the subsequent chapters and in which additional oedema-producing factors were used.

Another 10 anuric rabbits were giv en infusions of saline, 50—100 % of the body weight. Experiments with such a degree of overhydration do not seem to have been reported earlier

Moderate overhydration (Ringer s solution in amounts equalling 15 % of the body-weight)

Both ureters of 65 rabbits were tied After intervals of varying length, Ringer solution in amounts equalling 15 % of the body weight was infused I 1 1/2 hours (1 7 ml per min te and kg) Ringer solu tion contains 9.0 g of sodium-chloride and 0 24 g of calcium-chloride per 1000 ml. The rabbits were killed by bloodletting 2—4 or 24 hours after the infusion. X ray and electrocardiographic examinations were made and respiratory rate wa measured before the ligation of the ureters, before the infusion, and before the bloodl t ting, nd, in rabbits that were to be killed after 24 hours, immediately fter the infusion as well. The amount of solution for inf sion, 15 % of the body-weight, wa chosen to enable u t make comparison with earlier tudies (Alwall, Lunderquist, and others) and because it had been found that amounts of 25 % produced profuse pleural effusion, ascites, and telectasis which made the assessment f the chest radiographs difficult and also complicated the experimental conditions in other respects (Alwall & Lindqvi t)

The series was divided into 6 groups of 10—11 rabbits each, on the basis of dura tion of anuria and overhydration) Rabbits receiving the infusion 1/2 hour 24 hours, 48 hours, or 72 hours after the liga *tion of the ureters nd bled 2—4 hours* after the infusion b) Rabbits receiving the infusion 48 or 72 hours after the ligation f the ureters and bled after another 24 hours.

Appearance and behaviour The ani mals were not markedly affected by the infusion. Most of them became a little sluggish and sat still for a few hours after the infusion, but then they behaved just like other uraemic rabbits. In most of the highly uraemic rabbits the infusion led to general im provement and prolonged survival *times. Among 10 overhydrated rabbits* which at the time of infusion had been anuric for 72 hours, all survived an*other 24 hours*, whereas only 16 out of 49 non-overhydrated rabbits which had been anuric for 72 hours before infusion survived a further 24 hours (31 %)

The respiratory rate (Table 14) fell in most of the slightly uraemic rabbits after infusion of saline but remained unchanged in rabbits with severe urae-

mia One rabbit with pulmonary oedema had a respiratory rate of 84 per minute and dyspnoea. The highest rates, over 120 per minute, were re corded before bloodletting in 3 severely uraemic animals, including 1 with large amounts of pleural fluid and 2 with numerous haemorrhages in the lungs at autopsy and in 4 slightly uraemic rabbits without any pulmonary changes. During the first 24 hours following the infusion, the respiratory rate rose from 88 (70—120) to 105 (66—132) per minute in the group with anuria for 48 hours and fell from 83 (62—110) to 81 (62—102) per minute in the group with anuria for 72 hours

E C G and heart rate The heart rate showed a slight average rise during the infusion irrespective of the degree of uraemia (Table 14) During the first 24 hours after the infusion, the heart rate rose from 231 (200—269) to 232 (200—300) beats per minute in the group with anuria for 48 hours and fell from 263 (202—294) to 252 (178—385) beats per minute in the group with anuria for 72 hours

Electrocardiographic abnormalities did not occur either before or after the infusion in rabbits with anuria for less than 48 hours. In rabbits with anuria for at least 48 hours the electrocardiographic abnormalities improved during the infusion in 10 cases, became worse in 5 and remained unchanged in the rest. Thus 8 rabbits had before infusion deformed QRS-complexes and/or negative T waves after infusion 4 of these 8 animals had normal electrocardiograms in 1 the T wave was absent, in 1 negative, and 2 rabbits died during the infusion In the latter pulmonary oedema was not seen by gross examination at autopsy Within 24 hours of infusion the number of normal electrocardiograms in the group with anuria for 48 hours decreased from 9 to 8 and the number with absent T waves increased from 1 to 2 In the group with anuria for 72 hours the number of normal electrocardiograms decreased from 6 to 4 and the number of T wave flattening from 3 to 1 whereas that with negative T waves increased from 1 to 4 On one occasion the electrocardiograph was out of order

The heart size Before the infusion 12 % of the rabbits with anuria for at least 48 hours had cardiac enlargement, as against 0 % for those with anuria of shorter duration after infusion (Table 14) the figures were 44 % and 14 % respectively During the first 24 hours after the infusion the heart size in rabbits with anuria for 48 hours increased in 3 and remained unchanged in 7 In the group with anuria for 72 hours the heart size increased in 2 and remained unchanged in 8 cases Tables 12 and 13 show the heart size at the time of bloodletting

Chest radiography was before the infusion normal in all the rabbits excluding 1 This rabbit had ventricular fibrillation in the electrocardiogram cardiac enlargement (++) and slight pulmonary changes (+) before the infusion, but improved during the infusion the electrocardiographic evidence of ventricular fibrillation disappeared but the pulmonary changes and the

cardiac enlargement persisted (lung weight 5 67 g) After the infusion 5 rabbits with anuria for 48 hours and 1 rabbit with anuria for 72 hours had slight pulmonary changes (+) In 1 rabbit with anuria for 48 hours which lived for another 24 hours, the pulmonary changes disappeared. At the bloodletting slight changes were noted in 5 rabbits (Tables 12 and 13) Of these 5 animals, 1 had several other signs of slight pulmonary oedema 2 had profuse pleural effusion and atelectasis, 1 had atelectasis without pleural effusion, and in 1 no pulmonary changes were seen at autopsy but large amounts of ascitic fluid were found, and X ray showed cardiac enlargement.

Besides the afore-mentioned 2 animals that died during the infusion, one more died a few hours before the planned bloodletting and was found to have profuse pleural effusion and atelectatic lungs. The other 62 rabbits were killed by bloodletting (Tables 12 and 13)

Gross observation at autopsy 57 rabbits had pink and 5 had small bluish purple lungs. In 24 animals (including 19 which survived 24 hours after the infusion) the lung surfaces, notably at the base, showed numerous red dots, or brown spots no larger than confetti. Such dots or spots, marked by + in Tables 12 and 13, occurred abundantly in 4 animals of the groups with anuria for 48 and 72 hours. Rabbits which were bled 2 to 4 hours after the infusion and had had anuria for less than 72 hours exhibited no changes.

Tables 12 and 13 show that the average amount of ascitic fluid was greater in overhydrated than in non overhydrated animals. It increased with increasing uraemia and increasing length of post infusion survival. The individual variations were great however

Frothy fluid in the air ways 1 rabbit had frothy fluid in the trachea without any other signs of pulmonary oedema.

The lung weight per kg body weight in the 62 bled rabbits was 4.39 ± 0 49 g that is, largely the same as that in normal animals and controls (Table 7) It will be seen from Tables 12 and 13 that there was no difference in lung weight at different degrees of uraemia and different times of blood letting

The water content of the lungs It will be seen from Table 7 that the water content of the lungs of all the 62 bled rabbits was increased the mean being 82.2 ± 1.2 % that is, higher than in normal and control animals. The degree of uraemia made no difference (Tables 12 and 13). For rabbits bled 2—4 hours after the infusion, the average water content was *slightly* higher than for those bled 24 hours after the infusion.

Microscopical examination of the spots seen on the lung-surfaces revealed haemorrhages which in many cases were not *quite fresh*. In other respects the microscopical picture was virtually normal.

Pulmonary oedema and uraemia

Tables 12 and 13 show that pulmonary

Table 12 *Results after infusion of Ringer's solution amounting to the equivalent of 15 % of the body weight for 1 ½ hours in anuric rabbits whose ureters had been tied 0—1 24 48 and 72 hours respectively before the infusion The animals were bled within 2 to 4 hours of the infusion. L=at least three criteria of lung oedema.*

	Lung weight g/kg body weight	Water content %	Pulmonary oedema by gross observation	Frothy fluid in the airway	Alveolar oedema	X ray: Lung changes	X ray: Cardiac enlargement	Pleural fluid ml	Ascites ml	N P N mg per 100 ml
I. Infusion 0—1 hour after ligation of ureters	5.58	80.4	0	0	0	—	—	3	25	
	4.00	81.4	0	0	0	0	+	2	2	
	4.78	81.2	—	0	0	0	0	4	0	
	4.60	82.4	0	0	—	0	0	0	19	
	4.42	83.3	0	0	—	0	0	4	20	
	4.42	81.8	0	0	0	0	0	1	20	
	4.38	81.6	0	0	0	0	0	2	15	
	4.01	82.0	0	0	—	0	0	2	5	
	3.93	82.4	0	0	—	0	0	1	10	
	3.70	81.6	0	0	0	0	0	6	2	
	3.35	82.9	0	0	—	0	0	8	28	
Mean	4.38	82.0						4	13	51
II Infusion 24 hours after ligation of ureters	5.07	80.4	0	0	—	0	0	7	6	
	[illegible]	81.4	0	0	—	0	0	2	37	
	4.82	81.0	0	++	—	0	0	4	150	
	4.71	83.0	0	0	—	0	+	4	15	
	4.07	80.5	0	0	0	0	0	4	250	
	4.60	81.5	0	0	0	0	+	2	15	
	4.16	82.2	0	0	0	0	0	2	180	
	4.11	83.4	0	0	—	0	0	4	10	
	4.35	82.0	0	0	—	0	0	0	16	
	4.19	82.3	0	0	—	0	0	5	12	
Mean	[illegible]	81.8	0					3	60	75

III. Infection 48 hours after ligation of ureters	5.33	83.4	○	●	—	+	+	10	110	140
	5.16	82.2	○	○	—	○	+	7	80	230
	4.86	82.4	●	○	—	+	++	24	80	217
	4.80	83.6	○	○	—	○	+++	8	65	156
	4.57	84.1	●	○	—	●	+++	5	265	180
	4.24	83.8	○	○	—	●	+	13	38	150
	4.23	83.1	○	●	—	○	+	5	70	146
	4.14	82.7	○	○	—	●	○	2	130	162
	4.01	83.3	○	○	○	●	●	2	120	201
	3.08	82.0	●	○	●	○	○	2	40	190
	3.03	82.4	○	○	○	+	+	2	330	157
								6	120	174
Mean	4.41	83.2								
IV. Infection 72 hours after ligation of ureters L	5.67	87.0	+	●	○	+	++	9	74	250
	4.87	83.0	○	●	●	—	—	2	305	214
	4.53	83.1	○	○	○	○	○	1	220	272
	4.39	81.9	○	●	—	○	○	4	75	182
	4.12	82.1	○	○	—	○	○	2	141	256
	4.11	81.2	○	○	—	○	●	1	135	101
	3.98	81.8	○	○	●	○	●	1	270	210
	3.88	80.4	○	○	○	●	○	1	205	[illegible]
	3.53	82.8	●	○	—	○	+	0	32	262
	3.00	82.1	○	○	—	○	○	2	145	272
								3	150	210
Mean	4.30	82.4								

Table 13 *Results after infusion of Ringer's solution amounting to the equivalent of 15 % of the body weight for 1 ½ hours in anuric rabbits whose ureters had been tied 2 and 3 days respectively before the infusion The animals were bled within 24 hours of the infusion*

	B g weight kg body weight	Water content in %	Pulmonary oedema by gross observation	Frothy fluid in th (…)	Alveol oedema	X ray: Lung changes	X ray: Cardi enlargement	Pleural fluid ml	Ascites ml	N P N mg per 100 ml	Serum potas si m mEq/l
Infusion 48 ho rs afte lig ll n f uret rs	1.88	82.5	+	0	0	0	+	8	102	187	
	4.83	81.0	+	0	0	0	+++	6	92	283	
	4 8	82 0	0	0	0	0	+	7	165	102	
	4 77	81.8	0	0	0	0	+	5	32	246	
	4 71	81.2	0	0	0	0	++	30	55	250	
	4.20	82.0	0	0	0	+	++	50	200	227	
	4 17	80.5	0	0	0	0	0	23	130	210	
	1 14	81.9	+	0	0	0	++	7	73	—	
	3 87	81.0	+	0	0	0	0	7	30	204	
	[illegible]	81 7	0	0	0	0	0	4	250	150	
Mean	4 10	82 1						14 7	115	217	
Inf sion 72 hours aft r lig tion of ret rs	4.83	81.8	0	0	0	0	0	8	35	258	
	4 18	79.8	0	0	0	0	0	13	580	288	0 7
	4.23	81.5	+	0	0	0	+	0	56	257	8.8
	4 21	82.1	+	0	0	0	+	4	237	233	
	4.20	82.0	0	0	—	0	+	0	78	310	
	4 13	80.3	0	0	0	0	0	31	505	240	8.3
	4 10	80 1	0	0	0	0	0	16	110	246	
	3 93	80 4	+	0	0	0	0	0	3 0	230	8 5
	3.81	81 1	0	0	0	0	+	6	23	238	8.1
	3 2	82 1	0	0	0	0	++	8	[illegible]	240	6.0
Mean	4 15	81 18						10	213	250	

Table 14 *Respiratory rate heart rate and cardiac enlargement bef re and after infusion f Ringer s solution 15 % of the body-weight f r 1 f hours in anuric rabbits whose ureters had been tied 0—1 24 48 and 72 hours respectively befo e the infusi n*

Number of rabbits	Duration of anuria			
	3 hours	24 hours	48 hours	72 hours
	31	10	21	28
Respiratory rate per minute				
before infusion	100 (70—201)	143 (65—200)	102 (50—160)	73 (40—140)
after infusion	90 (43—206)	75 (29—140)	74 (37—120)	76 (48—110)
Heart-rate per minute				
before infusion	211 (130—321)	220 (194—280)	212 (117—283)	235 (178—300)
after infusion	250 (184—283)	224 (130—260)	221 (151—294)	247 (176—300)
Cardiac enlargement				
before infusion	0	0	3	2
after infusion	1	2	12	6

oedema, as defined here, arose after infusion of Ringer s solution in 1 of 10 rabbits which had had uraemia for 72 hours and were killed by bloodletting within 2—4 hours of the infusion. Pulmonary oedema did not develop in the groups that were overhydrated for 1 24 or 48 hours and bled within 2—4 hours of infusion, nor in the groups that were overhydrated for 24 or 72 hours after infusion and bled 24 hours later No tendency to storage of fluid in the lungs at high-degree uraemia was noted.

Criteria of pulmonary oedema

The animal with pulmonary oedema had slight radiographic pulmonary changes, slight macroscopical pulmonary changes, slightly increased lung weight and relatively greatly increased water content in the lungs. Among the rest of the 62 rabbits, 8 had increased water content, 7 had slight macroscopical changes, 4 had slight radiographic pulmonary changes, 2 had increased lung weight, 1 had frothy fluid in the air ways, but none had alveolar oedema.

Severe overhydration (Ringer's solution equalling 50—100 % of the body-weight)

Experiments were carried out with slow infusion of very large amounts of fluid in highly uraemic rabbits.

Five rabbits, anuric for 2 days, were given infusion of Ringer solution intravenously 50 % of the body weight, for 7 hours (1.2 ml per minute and kg) In 1 rabbit with negative T wave before infusion pulmonary oedema developed during the infusion. Three rabbits died spontaneously 7 8, and 23 hours after the completion of infusion, and 1 rabbit was bled 24 hours after infusion. These 4 rabbit had no pulmonary oedema but their lungs were telectatic. X-ray examination of the lungs howed marked indurations the water content was increased in only 1 rabbit, averaging 82.9 (79 7—85 7) %. none had frothy fluid in the ir ways the lung-

weights were normal, the average being 5.51 (4.80—6.58) g per kg body weight. Gross observation and microscopical examination showed no signs of oedema. The 4 rabbits had profuse pleural effusion 53 (30—102) ml and massive ascites, 394 (74—902) ml but little pericardial fluid.

Five uraemic rabbits, anuric for 3 days, were given Ringer's solution subcutaneously for 9 hours, 90—100 % of the body weight (1.7 ml per minute and kg). One rabbit died towards the end of the infusion, the others died 1, 6, 8 and 47 hours after completed infusion. None developed pulmonary oedema. X-ray of the lungs showed marked indurations. Autopsy immediately after death showed no frothy fluid in the air ways, small dark red lungs, normal (3 cases) or moderately increased (2 cases) lung weight, the average being 6.23 (5.10—7.70) g per kg body weight, normal water content in 4 cases and increased in 1 case, average 83.3 (81.4—86.5) %, little pleural fluid 6 (1—16) ml and pericardial fluid and large amounts of ascitic fluid 500 (132—815) ml. All the tissues seemed to be imbued with fluid. Microscopically atelectasis without oedema was seen.

The series was not completed as no pulmonary oedema, but only atelectasis, developed. These experiments showed that heavy overhydration of severely uraemic rabbits did not produce pulmonary oedema if the animal survived the infusion.

Infusion of mannitol solution

Mannitol has been used for assessment of the extracellular space in the body (62, 130, 188, 233, 234). Mannitol does not penetrate the red blood-cells and is not bound to protein. In man it has a smaller space (about 16 % of the body weight) than have chloride and sodium (about 23 % and 25 % respectively). Mulrow et al. (1956) found that nephrectomized dogs had a mannitol space of 22 % which after 30 hours had risen to 48 %. Last et al. (1952) observed that after infusion of mannitol intravenously 8 hours were required for the mannitol concentration in ascitic fluid and interstitial fluid to become equally high.

How large a part of mannitol is broken down has not been made clear. Dominguez (1947) found that in man 20 % is broken down and 80 % is excreted in the urine in 24 hours. According to Smith et al. (1940), 15 % and according to Schwarz et al. (1950) about 10 % is broken down in man. Elkinton (1949) found that the breakdown of mannitol in anuric patients is insignificant. Houch (1951) in dogs, observed no destruction of mannitol, since in 11 hours all of an injected quantity can be recovered in the urine. Carr et al. (1933, 1938) noted a slight rise of blood sugar in rabbits after oral administration of mannitol.

Side-effects of mannitol used clinically have occurred in the form of dyspnoea and pulmonary oedema in patients with cardiac failure (38), lower urinary-tract diseases (178) and renal insufficiency (21). Symptoms resembling those of acute water intoxication have also been described (222 a).

Barry & Berman (1961) observed that the blood volume of an anuric patient increased greatly during infusion of 100 ml of 20 % mannitol.

Marx et al. (1960) gave dogs intravenous infusions of fluid in amounts equalling 10 % of the body weight for 1 or 2 hours; one series received a solution of 5 % mannitol and 5 % sorbitol, and another series was given 5 % glucose. In a third series, a solution of 6 % dextran in 0.9 sodium chloride was infused for 1 hour in an amount corresponding to 5 % of the body weight in vivo. The blood volume increased with mannitol-sorbitol to the same extent as with glucose but not as much as with dextran. Pulmonary oedema was not noted.

When given in large doses to rats mannitol depresses cerebral function, just like other alcohols (Macht & Ting 1922).

Own investigations

To find out the frequency of pulmona ry oedema in uraemic rabbits at an in crease of the extracellular fluid, 5.5 % mannitol solution was infused intra venously in amounts equalling 15 % of the body weight for 5 hours.

The experimental conditions originally planned were the same s those in the saline experiments. Preliminary trials showed, however that uch a technique could not be used, because 3 animals which received infusions of 5.5 % mannitol solution intravenously 15 % of the body weight for 1 / hours, became se erely ill during or immediately after the infusion. Their breathing becam slow and irregular and their eyes large They died suddenly one of them after a drawn-out cry Pulmonary oedema was not seen t utopsy In this series, bloodletting was planned to take place 24 hours after the infusion, in the experiments with sa line. It will be seen from the following c count that the survival time was shorter in many cases. Bloodletting was therefore carried out when the animals had passed lat a clearly moribund state.

In 20 rabbits the ureters were tied bi laterally Two days later when the N.P.N level was around 180 mg per 100 ml, they were given infusions of isotonic (5.5 %) mannitol solution to which had been added 0.24 g of cal cium chloride and 0.42 g of potassium chloride per litre of fluid. The infused amount corresponded to 15 % of the body weight immediately before the infusion. The infusion time was 5 hours.

Among the 20 rabbits in the series, 1 died during the infusion, 1 immediately after the end of it, 6 within 12 hours, and 0 within 12 to 24 hours of the in fusion. Three survived 24 hours after the infusion.

The last mentioned 3 rabbits were bled 24 hours after the infusion. In all 10 rabbits were bled within 16 (15—24) hours of the infusion and 10 died spontaneously Of the latter only 5 could be examined promptly after death, while their bodies were still warm and soft in the other 5 cases rigor mortis had already set in at autopsy

Appearance and behaviour· The ani mals were sluggish trembling, and weak. Most of the time they lay flat on their abdomens, fore legs wide apart. They did not slaver and did not have exophthalmus. The corneal reflexes and nose-reflexes were normal. Dyspnoea rarely occurred. Most of the animals died quietly without convulsions.

The respiratory rate fell in the 19 rabbits that survived after the in fusion, on an average from 124 (76—176) before to 73 (36—133) per min ute after infusion, and then remained unchanged until death Only 1 animal, which had profuse pleural effusion (53 ml) was dyspnoeic.

E.C.G and heart-rate The heart rate rose in 19 rabbits, on an average from 226 (164—294) beats per minute before infusion to 257 (168—327) beats per minute after infusion. Immediately before the bloodletting the heart rate in 10 rabbits averaged 187 (88—308) beats per minute.

The electrocardiogram was normal before infusion in 17 out of 20 animals In 2 the T wave was isoelectric and in 1 negative. Immediately after

the infusion 7 out of 19 surviving animals had normal electrocardiograms, 4 had isoelectric T waves 7 had negative T waves, and 1 had a deformed QRS-complex. Immediately before the bloodletting 2 out of 10 surviving animals had normal electrocardiograms 5 had negative T waves and 3 had deformed QRS-complexes the electrocardiographic abnormalities had not disappeared in any of the animals since the infusion. Among those 5 that lived longest, 3 had normal electrocardiograms and 1 had isoelectric T wave immediately after the infusion.

The heart size assessed in the radiograph increased during the infusion in 9 of 19 animals (one died during infusion) The enlargement persisted or increased in 5 which were examined immediately before the bloodletting The size of the heart was not influenced by the infusion but remained normal until bloodletting in 4 animals which survived at least 18 hours after the infusion.

X ray evidence of slight pulmonary changes was noted in 2 of 19 rabbits by examination immediately after the infusion. It will be seen from Table 15 that immediately before the blood letting pulmonary changes were present in 5 animals Three of the latter had atelectasis resulting from profuse pleural effusion (46—62 ml)

Serum electrolyte concentrations after death The serum potassium concentration at the bloodletting averaged 7 4 (5 8—8 5) mEq per litre (6 animals) and the serum sodium concentration 103 (95—115) mEq per litre (5 animals)

Gross observation at autopsy Gross evidence of slight pulmonary oedema was seen in only 1 bled rabbit (Table 15) No such evidence was found in those that died spontaneously

The amount of pleural fluid averaged 18 (4—62) ml and that of ascitic fluid 100 (8—250) ml the subcutis was very rich in fluid

Frothy fluid in the air ways was not found in any animals.

The lung weight was raised in 2 bled rabbits. The average lung weight of the bled animals was 4.80 (4 0?—6 10) g of the spontaneously dead non-rigid animals 4 89 (3.80—6.30) g and of the spontaneously dead rigid ones 6 98 (6.20—8 12) g per kg body weight. In comparison with the control series the differences were insignificant

The water content of the lungs was slightly raised in 2 animals. The average water content for the bled animals was 82 4 (80 7—84 0) % which was somewhat higher than for the control series

Microscopical examination showed slight alveolar oedema in 1 rabbit Minor haemorrhages were seen in 4 In all the animals the alveolar septa were a little thicker than normally

Frequency of pulmonary oedema

Pulmonary oedema as defined here occurred in 1 of 10 bled animals but in none of the non rigid spontaneously dead ones As regards those examined after rigor mortis had set in the occurrence of mild degrees of pulmonary oedema cannot be assessed with certainty but oedema of high degree was definitely not present

Table 15. *Result ... fte ... l fusio ... of 5.5 % mannitol solution, 15 % ... f the body-weight f r 5 hours in anuric rabbit who e uret rs had been tied 2 day before the infusi n. The rabbit were killed by bloodletting. L=at least three ... rit ri ... f lung oedema*

Lung-weight g/kg body weight	Water content in lung %	Pulmonary oedema by gross observation	Frothy fluid in the air-	Alveolar oedema	X-ray: Lung changes	X-ray: Cardiac enlargement	Pleural fluid ml	Ascites ml	N.P.N. mg per 100 ml	Post infusion survival hours
6.10	81.0	+	0	+	++	+	9	64	253	6
5.52	82.8	0	0	0	—	—	9	53	222	11
5.28	83.2	0	0	0	+	+++	53	60	163	13
5.13	82.7	0	0	0	0	++	15	15	200	5
5.06	80.7	0	0	0	0	0	8	122	?04	18
4.55	80.4	0	0	—	0	0	8	210	373	18
4.49	82.2	0	0	0	0	0	4	145	215	21
4.26	82.8	0	0	—	+	+	19	95	236	21
4.15	81.3	0	0	0	++	0	46	256	220	23
4.02	81.8	0	0	0	+++	+	63	170	216	23
		0	0				23	118	220	18
Mean 4.86	82.4									

the infusion 7 out of 19 surviving animals had normal electrocardiograms 4 had isoelectric T waves 7 had negative T waves and 1 had a deformed QRS complex. Immediately before the bloodletting 2 out of 10 surviving animals had normal electrocardiograms, 5 had negative T waves and 3 had deformed QRS-complexes the electrocardiographic abnormalities had not disappeared in any of the animals since the infusion. Among those 5 that lived longest 3 had normal electrocardiograms and 1 had isoelectric T wave immediately after the infusion.

The heart size assessed in the radiograph increased during the infusion in 9 of 19 animals (one died during infusion) The enlargement persisted or increased in 5 which were examined immediately before the bloodletting The size of the heart was not influenced by the infusion but remained normal until bloodletting in 4 animals which survived at least 18 hours after the infusion.

X ray evidence of slight pulmonary changes was noted in 2 of 19 rabbits by examination immediately after the infusion It will be seen from Table 15 that immediately before the bloodletting pulmonary changes were present in 5 animals. Three of the latter had atelectasis resulting from profuse pleural effusion (40—62 ml)

Serum-electrolyte concentrations after death The serum potassium concentration at the bloodletting averaged 7 4 (5 8—8 5) mEq per litre (6 animals) and the serum sodium concentration 103 (95—115) mEq per litre (5 animals)

Gross observation at autopsy Gross evidence of slight pulmonary oedema was seen in only 1 bled rabbit (Table 15) No such evidence was found in those that died spontaneously

The amount of pleural fluid averaged 18 (4—62) ml and that of ascitic fluid 100 (8—250) ml the subcutis was very rich in fluid.

Frothy fluid in the air ways was not found in any animals

The lung weight was raised in 2 bled rabbits. The average lung weight of the bled animals was 4 86 (4 0?—6 10) g of the spontaneously dead non rigid animals 4.89 (3.80—6 30) g and of the spontaneously dead rigid ones 6 98 (6.20—8 12) g per kg body weight. In comparison with the control series the differences were insignificant

The water content of the lungs was slightly raised in 2 animals. The average water content for the bled animals was 82 4 (80 7—84 0) % which was somewhat higher than for the control series

Microscopical examination showed slight alveolar oedema in 1 rabbit Minor haemorrhages were seen in 4 In all the animals the alveolar septa were a little thicker than normally

Frequency of pulmonary oedema

Pulmonary oedema as defined here occurred in 1 of 10 bled animals but in none of the non rigid spontaneously dead ones. As regards those examined after rigor mortis had set in the occurrence of mild degrees of pulmonary oedema cannot be assessed with certainty but oedema of high degree was definitely not present

blood sugar concentration immediately after the infusion averaged 0 79 (0 72—0.87) g per 100 ml.

After the infusion, the animals first improved temporarily and then became increasingly sluggish, mostly lying motionless flat on their abdomens, seemingly exhausted, and walking with an unsteady gait. Nystagmus was noted in many cases. Muscular twitchings occurred in different extremities or muscle groups. The saliva flowed. Their condition was as a rule worst 6—8 hours after the infusion. The blood-sugar concentration of 9 cases averaged 0 19 (0 11—0.33) g per 100 ml about 6 hours after the infusion whereas in 1 it was still 0 70 g per 100 ml 6 hours after the infusion. Nine rabbits were bled at this stage, as their general condition was very poor (Table 16) At the bloodletting the serum contained, on an average, 105 (89—113) mEq of sodium and 0.9 (5.6—7.9) mEq of potassium per litre

From 8 hours after infusion up to the bloodletting 24 hours after infusion many of the animals improved. Most of them were still very weak, however and 5 died spontaneously during this period. At the bloodletting 24 hours after the infusion (10 rabbits) N.P N averaged 204 mg per 100 ml (Table 10) The serum sodium was 111 (98—120) and serum potassium 0 7 (5.2—7 7) mEq per litre (8 and 5 rabbits, respectively) The blood-sugar was normal in 2 animals.

The respiratory rate fell during the infusion, from an average of 126 (72—138) to an average of 0 (28—120) per minute. In 9 moribund dogs, about 6 hours after infusion, the respiratory rate averaged 00 (20—110) per minute at the bloodletting, and in 10 animals 24 hours after infusion it averaged 78 (51—118) per minute.

E.C.G and heart rate The heart rate changed on an average very little during and after the infusion.

Electrocardiography showed slightly flattened T waves, but only 1 rabbit had negative T wave after the infusion. About 6 hours after the infusion 9 moribund rabbits had abnormal electrocardiograms, showing isoelectric T waves in 3 negative T waves in 4 and serious deformation of the QRS-complexes in 2 Among 10 rabbits which survived until the bloodletting 24 hours after the infusion the electrocardiograms were still normal in 8, whereas 2 had negative T waves.

The heart size increased during the infusion to ++ in 3 rabbits and to + in 7 whereas it remained unchanged in 13 The examination was not successful in 1 rabbit. At the bloodletting 2 animals had cardiac enlargement, in 3 which had cardiac enlargement immediately after the infusion, the heart was of normal size.

Slight radiographic pulmonary changes were present in 1 of 23 rabbits after the infusion and persisted at the bloodletting 24 hours later No pulmonary changes were noted in the other rabbits. At autopsy 45 ml of pleural fluid were found in 1 rabbit although X ray immediately before death had not shown any abnormalities, mistake?

Gross observation at autopsy All the tissues (subcutis, viscera etc.) appear

Criteria of pulmonary oedema

The rabbit with "pulmonary oedema" had all the other criteria but lacked frothy fluid in the air ways. With the exception of positive findings as regards chest X ray in 4 and lung weight and water content in 1 all the criteria were negative in the other 9 bled animals

Increase of all the fluid spaces

Infusion of glucose solution

Cohnheim & Lichtheim (1877) induced pulmonary oedema in dogs and rabbits by infusing very large amounts of 3 % glucose solutions intravenously Fremont Smith & Forbes (1927) showed that intravenous infusion of hypotonic glucose solutions in animals caused a marked rise of the intracranial pressure. Warthen (1935) did not note pulmonary oedema after intravenous infusion averaging as much as 134 ml of 5 % glucose solution per kg of body weight for only 24 minutes. The blood-sugar rose, on an average from 124 mg to 454 mg per 100 ml. Infusion of 10 % solution caused fatal cerebral oedema in 30 % of the animals within a few hours Yeomans et al. (1943) did not note pulmonary oedema after infusion of 150—300 ml of 5 % glucose solution per kg body weight and hour Lands et al. (1940) found that the larger part of infused 5 % glucose solution accumulated in the extracellular space All the experiments quoted here were made on animals with intact renal function

In 1911 glucose infusions were introduced by Matas in human therapy Altschule et al. and Gilligan et al. (1938) noted a rapidly transient fall of haematocrit and serum protein values and an increase of the plasma and blood volumes after intravenous infusion of glucose. Hypertonic glucose solutions increased the blood volume slightly more than did isotonic glucose solutions. Murphy et al. (1941) observed the same increase of the plasma volume and the venous pressure after infusion of 5 % glucose solution and the same amount of saline, respectively

Funck Brentano et al. (1950) found that administration of large amounts of saline through a gastric tube causes no symptoms in rabbits but that the same amount of water produces fatal cerebral oedema They considered that cerebral signs of water intoxication in rabbits are due less to the increase of the total fluid than to the expansion of the intracellular volume

Own investigations

This series of experiments were designed to find out whether an increase of the fluid content in the body without a corresponding increase (relative decrease) of the body's salt content would produce pulmonary oedema in uraemic rabbits.

In 28 rabbits both ureters were ligated. Two days later the NPN level averaged 180 mg per 100 ml Isotonic glucose solution (5 5 %) to which had been added 0 24 g of calcium chloride and 0 42 g of potassium chloride in a volume equalling 15 % of the body weight was infused intravenously for 1 1/2 hours.

Appearance and behaviour During the infusion the rabbits were sluggish sat still most of the time and were slow in reacting to stimuli Marked exophthalmus was noted Towards the end of the infusion many of them bent their heads back. At times they were restless and had convulsive twitchings 4 rabbits died with convulsions Only a few had dyspnoea In 9 cases the

blood sugar concentration immediately after the infusion averaged 0.79 (0.72—0.87) g per 100 ml.

After the infusion, the animals first improved temporarily and then became increasingly sluggish, mostly lying motionless flat on their abdomens, seemingly exhausted, and walking with an unsteady gait. Nystagmus was noted in many cases. Muscular twitchings occurred in different extremities or muscle groups. The saliva flowed. Their condition was as a rule worst 6—8 hours after the infusion. The blood-sugar concentration of 9 cases averaged 0.19 (0.11—0.33) g per 100 ml about 6 hours after the infusion, whereas in 1 it was still 0.70 g per 100 ml 6 hours after the infusion. Nine rabbits were bled at this stage, as their general condition was very poor (Table 16). At the bloodletting the serum contained, on an average, 105 (89—113) mEq of sodium and 6.9 (5.6—7.9) mEq of potassium per litre.

From 8 hours after infusion up to the bloodletting 24 hours after infusion many of the animals improved. Most of them were still very weak, however and died spontaneously during this period. At the bloodletting 24 hours after the infusion (10 rabbits) N.P.N. averaged 204 mg per 100 ml (Table 16). The serum sodium was 111 (98—120) and serum-potassium 6.7 (5.2—7.7) mEq per litre (8 and 5 rabbits, respectively). The blood-sugar was normal in 2 animals.

The respiratory rate fell during the infusion, from an average of 126 (72—138) to an average of 70 (28—120) per minute. In 9 moribund dogs, about 6 hours after infusion the respiratory rate averaged 69 (20—110) per minute at the bloodletting and in 10 animals 24 hours after infusion it averaged 78 (51—118) per minute.

E.C.G. and heart rate. The heart rate changed on an average very little during and after the infusion.

Electrocardiography showed slightly flattened T waves, but only 1 rabbit had negative T wave after the infusion. About 6 hours after the infusion 9 moribund rabbits had abnormal electrocardiograms, showing isoelectric T waves in 3, negative T waves in 4 and serious deformation of the QRS-complexes in 2. Among 10 rabbits which survived until the bloodletting 24 hours after the infusion, the electrocardiograms were still normal in 8, whereas 2 had negative T waves.

The heart size increased during the infusion to ++ in 3 rabbits and to + in 7 whereas it remained unchanged in 13. The examination was not successful in 1 rabbit. At the bloodletting 2 animals had cardiac enlargement. In 3 which had cardiac enlargement immediately after the infusion the heart was of normal size.

Slight radiographic pulmonary changes were present in 1 of 23 rabbits after the infusi n and persisted at the bloodletting 24 hours later. No pulmonary changes were noted in the other rabbits. At autopsy 45 ml of pleural fluid were found in 1 rabbit, although X ray immediately before death had not shown any abnormallities mistake?

Gross observation at autopsy. All the tissues (subcutis viscera etc.) appear

ed to be very rich in fluid The brain was *not examined.*

Only 1 out of 4 rabbits that died in connection with the infusion had signs of pulmonary oedema at autopsy namely a white-spotted (+) lung surface *a lung weight of* 7 75 g per kg body weight, and a water content in the lungs of 84 5 % frothy fluid in the air ways was not seen.

Five rabbits, which died spontaneously *1 –23 hours after the infusion*, had large amounts of pleural fluid. In *1* animal autopsy immediately after death showed no evidence of pulmonary oedema the lung weight was 4 18 g per kg The other rabbits could not be examined until rigor mortis had occurred. In 3 of them pulmonary oedema was not seen by gross observation and in 1 the picture could not be assessed

One out of 19 bled animals had macroscopical evidence of slight pulmonary oedema (Table 16) in the form of numerous red dots and white spots. Five rabbits had, in addition, a few small haemorrhages and 4 had bluish *collapsed lungs* whereas 9 had normal lung surfaces.

Frothy fluid in the air ways was not seen in any animal.

The lung weight in the bled animals was increased in 1 out of 19 rabbits (Table 16) this animal had slight hyperaemia and minor *haemorrhages* established microscopically The average lung weight was 4 46 (3 74—6 87) g per kg body weight which is only slightly higher than in the control series

The water content of the lungs was slightly increased in 4 out of the 19 bled rabbits in Table 16 The averaged content was 82.2 (80 1—84 0) %, which is somewhat higher than in the control series.

Microscopical examination showed alveolar oedema in only 1 rabbit. Haemorrhage and atelectasis were seen in rabbits with macroscopically observed dots and bluish lungs, respectively

Frequency of pulmonary oedema

A sufficient number of criteria of pulmonary oedema as defined here were not found in any rabbit after the infusion.

Criteria of pulmonary oedema

Among the 19 bled animals, 4 had an increased water content in the lungs. Radiographic pulmonary changes occurred in 1 macroscopical and microscopical evidence of pulmonary oedema was noted in 1 each and increased lung weight in 1 Frothy fluid in the air ways was not present in any animal

Infusion of urea solution

Urea is distributed in a space approximating to that of the total fluid (201 203 235) and equilibrium between the concentrations in different organs is reached within an hour Rolls (1943) however found that the concentration of urea is higher in red cells than in plasma.

Vanquelin & Segales (182?) seem to have been the first to give urea to animals *Streicher* (1928) injected 200 ml of 10—20 % urea intravenously in dogs with normal renal function. The concentration of urea in the blood rose maximally to 700

Table 18. R sult aft infusion f 5.5 % glucose soluti n amounting to the equivalent f 15 % f the body-weight f r 1 1/ hours in anuric rabbits whose ureters had been tied 2 d ys bef re th infusi n. The rabbits were killed by bloodletting.

	Lung-weight g/kg body-weight	Water content l lung %	Pulmonary oedema by gross observation	Frothy fluid in the air-way	Alveolar oedema	X-ray: Lung changes	X-ray: Cardiac enlargement	Pleural fluid ml	Ascites ml	N. P. N. mg per 100 ml	Post-infusion survival hours
	5.87	81.0	●	0	●	—	—	4	64	180	5
	5.08	82.3	0	0	—	0	0	3	23	113	6
	4.93	82.6	●	●	+	0	0	1	118	101	6
	4.81	80.7	0	0	●	●	0	2	139	175	21
	4.74	80.1	0	●	—	0	0	0	305	277	21
	4.71	83.4	0	0	●	+	++	5	32	108	24
	4.63	82.8	●	●	●	●	0	9	8	104	26
	4.58	83.6	+	●	0	—	—	3	33	193	6
	4.54	82.5	●	0	●	0	0	0	37	161	6
	4.37	82.8	0	●	0	—	—	28	52	142	7
	4.36	80.7	●	0	—	0	0	4	287	174	21
	4.21	81.8	0	0	—	0	0	2	120	192	21
	4.11	81.7	0	●	0	0	+	0	15	198	6
	4.10	84.0	●	0	—	—	—	11	63	174	6
	4.08	83.0	0	0	—	0	0	14	113	108	21
	4.07	82.6	●	0	0	—	—	4	28	170	6
	4.06	82.0	●	0	—	0	0	2	300	197	21
	3.83	81.5	0	0	—	0	0	0	79	256	21
	3.74	80.4	0	●	0	0	0	45	221	192	21
Mean	4.46	82.2						8	109	181	16

mg per 100 ml Vomiting occurred at 200 mg dyspnoea and restlessness at 300 mg tremor after 400 mg convulsions after 500 mg, and diarrhoea unconsciousness, and death after 600 mg per 100 ml No signs of haemolysis were noted. The blood pressure rose markedly Autopsy of the lungs showed hyperaemia but no pulmonary oedema. Similar results were obtained by Grollman & Grollman (1959) in nephrectomized dogs in which the urea concentration was kept constant by peritoneal dialysis. Mayerson et al (1962) observed increased permeability in the lymphatics at high urea levels (at least 8 %) Schiano & d Onofrio (1957) found that in rabbits whose ureters had been tied the N.P.N level averaged initially 35 mg, after 24 hours 172 mg and after 52 hours 275 mg per 100 ml. Injection of 200 mg of urea per kg body weight (the concentration of the solution is not stated) led to death within a few hours.

Creevy (1951) noted marked haemolysis at irrigation of the bladder in man, using 1.8 % urea in aqueous solution Roberts (1955) showed that aqueous solutions of urea promptly produced haemolysis both in vivo and in vitro in diluted as well as in concentrated solutions. If 25 % urea was added to isotonic solutions of glucose or sodium chloride haemolysis did not occur

Fremont Smith & Forbes (1927) showed that intravenous injection of 50 % urea solution causes a marked fall of the intra cranial pressure Javid & Andersson (1959) found that hypertonic urea solution lowers the intracerebral pressure in bilaterally nephrectomized monkeys Alexander et al (1961) noted a great increase of the plasma volume in dogs after intravenous infusion of 30 % urea in 10 % invertose solution.

Own investigations

The purpose of this experimental series was to find out whether overhydration and a high concentration of urea by administration of urea solutions, would produce pulmonary oedema in anuric rabbits.

The ureters were tied in 42 rabbits weighing between 2 20 and 2 40 kg. Sterile urea powder was dissolved in a sterile fluid to the desired concentration immediately before the infusion.

The animals in the first series which had been anuric for 48 hours, died during the infusion of isotonic (1 8 %) urea solution in water for 1 ½ hours, in an amount equalling 15 % of the body weight The technique was therefore varied as follows Longer infusion time, hypertonic solutions different solvents different lengths of the interval between ureteral ligation and infusion and subcutaneous instead of intravenous injection Irrespective of technique the rabbits became severely ill after the infusion. The desired combination of fluid retention and high urea concentration could be carried through only for a short time

a) Intravenous infusion of 1.8 % urea solution in an amount corresponding to 15 % of the body weight for 1 ½ hours in 4 rabbits which had been anuric for 48 hours.

During the infusion signs of restlessness appeared the animals swallowed frequently and their tongues became bluish Sluggishness and exophthalmus were not noted All the animals died during the infusion in convulsions Autopsy showed no evidence of pulmonary oedema but blood serum was red in colour after centrifugation (haemolysis)

b) Intravenous infusion of 1 8 % urea solution in an amount corresponding to 15 % of the body weight for 3 hours in 4 rabbits which had been anuric for 48 hours.

The reactions of the animals were the same as in the previous series. 2 rabbits died during the infusion, 1 died 3 hours later whereas 1 survived 24 hours, alert but slightly trembling, and with an N.P.N level of 344 mg per 100 ml. The latter was killed by bloodletting. Thi rabbit and the one that died spontaneously after 3 hours had no pulmonary oedema. Aut psy after 1 / hours of the two that died during the infusion showed slight increase of the weight and water content of the lungs but no frothy fluid in the air ways. One of them had slight radiographic pulmonary changes immediately before death and the ther had slight macroscopical signs of p lmonary oedema.

c) Subcutaneous infusion of 1.8 % urea solution in an amount equalling 15 % of the body weight. The volume was divided into five doses which were infused at one-hour intervals. The series comprised 10 rabbits which had been anuric for 48 hours.

One rabbit died sponta eously d ring the inf sion and 6 died within 2 to 18 hours of the infusion. 3 rabbits, which were all 19—24 hours fter the infusion, were kil led by bloodletting. The clinical picture after infusion was similar in all the animal They showed lack of strength, trembled and twitched but reacted to li m li the sali flowed from their mouths, they lay with thei heads resting on the floor between their paws. Ny tagmus and exophthalmus were not oted. Death oc curred unnoticeably without convulsions, dy pnoea, etc. The respiratory rate fell, on an erage from 142 to 110 per minute during the infusion. Finally breathing became increasingly lower and weaker. 6 of the 9 rabbit that urvi ed the infusion had deformed QRS-complexes or negativ T w ves afterwards, and in 2 of the rest uch hanges developed later. All those that died pontaneousl were w rm and soft. t topsy I 4 of these cases utopsy was performed within 15 minutes and in the other 3 within 60 to 80 minutes of death. 2 of the latter had the highest lung weights in the series. At autopsy N.P.N veraged 354 (302—396) mg per 100 ml. A will be seen in Table 17 all the criteria of pulmonary oedema were lacking.

(d) Subcutaneous infusion of 1.8 % urea solution in amounts equalling 15 % of the body weight The volume was divided into five doses which were infused at one-hour intervals. The series comprised 4 rabbits which had been anuric for 1 hour

All the 4 animals remained lively during and after the infusion up to the bloodlet ting 24 hours later. E.C.G. emained nor mal. N.P.N at the bloodletting averaged 280 (264—294) mg per 100 ml. All the cri teria of pulmonary oedema were lacking. The lung weight a eraged 4.26 (4.06—4.43) g, the water content 82.4 (82.1—82.6) %, the pleural fluid 0.5 (0—1) ml, and the ascitic fluid 5 (0—10) ml. The lungs looked quite normal.

(e) Subcutaneous and intravenous in fusion, respectively of 1.8 % urea in 5.5 % glucose solution in amounts equalling 15 % of the body weight for 4 hours. Each series comprised 2 animals which had been anuric for 48 hours.

Exophthalmus, irregular breathing, tag gering gait, and weakness were noted in all the animals, most ma kedly in the intravenous-inf sion group. All had severe electrocardiographic changes t the end of the infusion. The rabbits in the intra enous-infusion group died shortly after the infusion and those in the subcutaneous-infusion group within about 6 hours f the infusion. X ray and autopsy show ed n evidence of pulmonary oedema.

Table 17 *Results after infusion of 1.8 % urea solution subcutaneously 15 % of the body-weight for 4 hours in anuric rabbits whose ureters had been tied 2 days before the infusion S=spontaneous death*

	Lung weight g/kg bod weight	Water content l lung %	Pulmonary oedema by gross observation	Frothy fluid in the ai way	Al eolar oedem	X-ray Lu g ha ges	X-ray Cardiac enlargement	Pleural fluid ml	Ascites ml	N. P. N mg per 100 ml	Post-infusion survival hours	
	6.37	80.1	0	0	—	—	—	0	0	—	0	S
	0.07	82.3	0	0	—	—	—	0	0	344	5	S
	5.81	82.6	0	0	—	—	—	4	2	325	7	S
	5.57	80.7	0	0	—	—	—	0	0	326	4	S
	5.14	80.8	0	0	0	—	—	0	45	370	18	S
	5.08	82.3	0	0	—	0	0	0	2	302	2	S
	4.98	83.1	0	0	—	0	+	5	75	370	20	
	1.02	81.8	0	0	0	0	0	0	20	383	10	
	1.82	81.2	0	0	—	0	+	0	11	352	7	S
	4.01	80.8	0	0	0	0	0	8	233	300	24	
Mean	5.28	81.6						2	39	354		

(f) Subcutaneous or intravenous infusion of 1.8 % urea in 10 % invertose solution in amounts equalling 10—15 % of the body weight for 1—3 hours The series comprised 12 rabbits which had been anuric for 48 hours.

(1) 6 rabbits received a volume of 15 % of their body weights intravenously for 3 hours

All the animals became very poor lying still, flat on their abdomens nystagmus was noted in many cases, dyspnoea in none E.C.G showed severe changes. The animals died quietly during or shortly after the infusion, excluding one which survived 6 hours.

(2) 3 rabbits received amounts equalling 10 % of their body weights intravenously for 1 hour and 3 the same amounts subcutaneously

2 were killed by bloodletting after 24 hours, and 4 died spontaneously after 2—12 hours of increasing weakness. In the intravenous-infusion group E.C.G. showed severe changes the animals in the subcutaneous-infusion group had no E.C.G changes and they did not survive longer Autopsies showed none of the criteria of pulmonary oedema.

(g) Intravenous infusion of 30 % urea in 10 % invertose solution in amounts equalling 1.3 % of the body weight for 30 minutes The series comprises 4 rabbits which had been anuric for 1 hour

1 animal had trembling after the infusion, the others were unaffected N E.C.G. changes appeared immediately after the infusion. 2 rabbit died spontaneously within about 20 hours 2 were bled after 24 hours, one of them in a very poor general condition. N.P.N averaged 380 (362—390) mg per 100 ml. Autopsies showed none of the criteria of pulmonary oedema.

(h) In 2 rabbits extremely severe pulmonary oedema developed after infusion of 20 ml of 50 % urea intravenously for 2—4 minutes.

Toxicity of urea

Infusion of isotonic urea solution in amounts equalling 10—15 % of the body weight had a toxic effect on moderately uraemic rabbits, whether the solvent was water 5.5 % glucose, or 10 % invertose, and even with slow subcutaneous infusion. Rabbits with slight uraemia, on the other hand, tolerated slow subcutaneous infusion of the same amount of isotonic urea solution. Anuric rabbits died at N P N levels of 300—400 mg per 100 ml even when they were not overhydrated and the rise in N.P N was largely attributable to urea.

Frequency of pulmonary oedema

Pulmonary oedema as defined here occurred in 2 animals which were given 50 % urea solution by rapid intravenous infusion but did not occur in animals which were given isotonic urea solution or 30 % urea solution for 30 minutes. A few criteria of pulmonary oedema were present in animals that died during the infusion.

Survey See page 146

CHAPTER 7

Pulmonary oedema following anaesthesia induced with barbiturates

Moon & Morgan (1936) noted pulmonary oedema in dogs which had received repeated large doses of phenobarbitone intravenously for several days. Autopsies revealed petechial haemorrhages on the lung surfaces and the lungs were filled with blood and oedematous areas of bronchopneumonia were also seen. The oedema was considered to be a result of increased permeability in the lung capillaries caused by prolonged shock. Gordh (1945) reported pulmonary oedema of 3 rabbits in connection with vagal apnoea, which was produced under deep narcotal narcosis (sodium isopropyl B bromallyl N methyl malonylcarbamide). Alwall et al. (1949) had technical difficulties at dialysis of rabbits by means of the artificial kidney because many of the animals, which were treated under Narkotal anaesthesia, died from pulmonary oedema. An analysis of the experimental conditions showed that the anaesthesia was the cause of the complicating lung oedema. Stone & Loew (1949) found that adrenaline produced severe pulmonary oedema in rabbits which were pretreated with phenobarbitone. Barriety & Kohler (1953) and Kohler & Barbe (1954) described pulmonary oedema in rabbits following intravenous injection of the barbiturate compound 'Somnifene'. Pulmonary oedema arose only in those animals which received such large doses that they died. Curare, ganglion blocking agents, and, to a smaller extent, atropine increased the tendency to pulmonary oedema after barbiturates.

Durlacher et al. (1950) found that post mortal lung oedema could be induced with Nembutal® (sodium ethyl-(1 methylbutyl) malonylcarbamide) (see Chapter 4).

Experiments have also been reported in which barbiturates were capable of protecting animals against pulmonary oedema.

Brunn (1933) induced pulmonary oedema in vagotomized rabbits by rapid intravenous infusion of saline solution. Pretreatment with 0.1 g of phenobarbitone per kg body weight prevented the development of pulmonary oedema. Lulsada & Sarnoff (1946) noted a similar effect of phenobarbitone in experiments on dogs. Poulsen (1946) found that aprobarbitone protected mice against lung oedema from inhalation of CO_2.

Under the experimental conditions applied in this investigation the animals developed anoxia and a survey of the literature on the subject will therefore be of interest.

Nairn (1951) on the basis of the relevant literature and his own experiments concluded that acute anoxia increases capillary permeability in extremely severe cases only and that there is no evidence that a long standing mild hypoxia would increase the susceptibility to oedema. Courtice & Korner (1952) were however able to induce pulmonary oedema in hypoxic rabbits by infusion of Ringer Locke solution.

Each by itself neither hypoxia nor infusion p oduced pulm nary oedema. Pattle (1956) e posed one lung of an a imal t anoxia while the other one wa normally oxygenated. After pulmonary oedema had been induced in the nimal (with adrenaline ammonia, phosgen) he could not demonstrate greater usceptibility to oedema on the anoxic side. Poulsen (1956) was not able to ind ce p lmonary oedema in mice unless the oxygen concentration in the inspired air was as low as 5 %. Poulsen (1951 1956) noted low frequency of pulmonary oedema in mice which had inspired 10—15 % CO_2 fo 5 min tes and a low frequency of severe pulmonary oedema fter inspiration of 25 % or higher concentrations. Rabbit also devel ped pulmonary oedema after inhalation of CO_2, which potentiates the anaesthetic action of barbiturates.

The f llowing authors deal with some other technical problems which are of interest here

Gordh (1945) found that the depth of narcosis induced by Narkol l in rabbit can be graded according to the scending intercostal paralysis. H lso showed that lowering of the head end could prevent pnoea t admini tration of doses that ca sed pnoea when the head end wa slightly raised. The heart wa dilated during the narcosis. Dundee & Richard (1951 observed that penthotal-sodium i metabolized lower i raemic tha by normal animals, and that the susceptibility to penthotal-sodium increases in raemic nimals.

NarLotal —uraemia—fluid retention l ng

To enabl a tudy of the pathogenesi of radiographic pulmonary changes designated s uraemic lung uraemic pulmonary oedema etc., Alwall, at the end f the 1940s, tarted experiments on rabbits, designed to provoke slowly developing radiographic pulmonary changes of the afore mentioned type (Alwall 1952 1954 Alwall & Lunderquist 1953 Lunderquist 1953 Alwall et al. 1963)

The rabbits ureters were tied under local anaesthesia. Immediately after the operation, Ringer s solution in amount equalling 10 15, and 25 % of the animals body weight was infused intravenously for 1 / hours. The animals were X rayed daily i the erect and the lateral position for the presence of pulmonary changes and pleu ral effusion, respecti ly In the series of 10 % Ringer s solution no pulmonary changes were noted in the series f 15 % Ringer s solution pulmonary cha ges occurred in 24 % of the cases, but they were only slight and did not ppear until the 2nd post inf sion day later The frequency was thu too low and the survival time (observation period) f r the uraemic a imals too hort. It wa not possible to tudy as pla ned, the effect of ltrafiltration of the blood in vivo for the purpose f removing the administered amount of fluid, that is, the effect of dehydration on the pulmonary changes. In the series of 25 % Ringer s solution later on enlarged in co-operation with the present uthor radiographic pulmonary cha ges ppeared sooner or later in half to two-thirds of the nimals, but the film were difficult to asses because of profuse pleural effusion.

I 1949 the experimental technique wa therefore modified a follows, on the basis of the observation that N rkotal' narcosi produces pulmonary oedema in rabbit The ureters were tied under 'N rkotal' narcosi : 1.2 ml of 'Narkotal' solution per kg bod weight wa injected for 20 minutes The narcosis wa of sufficient depth and duration t llow painless ligation of the ureters nd exposure of the jugular vei for insertion of a cann la through which Ringer solution was infused for

CHAPTER 7

Pulmonary oedema following anaesthesia induced with barbiturates

Moon & Morgan (1936) noted pulmonary oedema in dogs which had received repeated large doses of phenobarbitone intravenously for several days. Autopsies revealed petechial haemorrhages on the lung surfaces and the lungs were filled with blood and oedematous areas of bronchopneumonia were also seen. The oedema was considered to be a result of increased permeability in the lung capillaries caused by prolonged shock. Gordh (1945) reported pulmonary oedema of 3 rabbits in connection with vagal apnoea, which was produced under deep narcotal narcosis (sodium isopropyl B bromallyl N methyl malonylcarbamide). Alwall et al. (1949) had technical difficulties at dialysis of rabbits by means of the artificial kidney because many of the animals, which were treated under Narkotal anaesthesia, died from pulmonary oedema. An analysis of the experimental conditions showed that the anaesthesia was the cause of the complicating lung oedema. Stone & Loew (1949) found that adrenaline produced severe pulmonary oedema in rabbits which were pretreated with phenobarbitone. Bariety & Kohler (1953) and Kohler & Barbe (1954) described pulmonary oedema in rabbits following intravenous injection of the barbiturate compound Somniféne. Pulmonary oedema arose only in those animals which received such large doses that they died. Curare, ganglion blocking agents, and, to a smaller extent atropine increased the tendency to pulmonary oedema after barbiturates.

Durlacher et al. (1950) found that post mortal lung oedema could be induced with Nembutal ® (sodium-ethyl-(1 methylbutyl) malonylcarbamide) (see Chapter 4).

Experiments have also been reported in which barbiturates were capable of protecting animals against pulmonary oedema.

Brunn (1933) induced pulmonary oedema in vagotomized rabbits by rapid intravenous infusion of saline solution. Pretreatment with 0.1 g of phenobarbitone per kg body weight prevented the development of pulmonary oedema. Luisada & Sarnoff (1946) noted a similar effect of phenobarbitone in experiments on dogs. Poulsen (1945) found that aprobarbitone protected mice against lung oedema from inhalation of CO_2.

Under the experimental conditions applied in this investigation the animals developed anoxia and a survey of the literature on the subject will therefore be of interest.

Nairn (1951) on the basis of the relevant literature and his own experiment concluded that acute anoxia increases capillary permeability in extremely severe cases only and that there is no evidence that a long standing mild hypoxia would increase the susceptibility to oedema. Courtice & Korner (1952) were however able to induce pulmonary oedema in hypoxic rabbits by infusion of Ringer Locke solution

injected intravenously into an ear vein, 1 7 ml per kg body weight the animals were weighed immediately before the induction of narcosis. The injection was made as rapidly as was possible without killing the animal. Deep narcosis was desired, with momentary respiratory standstill or a few minutes of bradypnoea (18 respirations per minute or less)

The doses of 'Narkotal were thus larger than those used by Alwall et al., who gave 1.2 ml per kg body weight to induce narcosis with abolition of pain for 20 minutes. In these experiments the rate of injection was adjusted to the respiratory rate and deep narcosis was aimed at (18 espirations per minute, or lower preferably without respiratory standstill) The higher dose 1 7 ml, instead of 1.2 ml, per kg body weight was established after pilot experiments using Alwall s et al. technique) 8 rabbits which had been anuric for 24 hours were anaesthetized with 1.2 ml f Narkotal per kg body weight. Ringer solution was then infused in amounts equalling 15 % of the body weight for 1 / hours. The animals were bled 1 hour later In 2 of the cases pulmonary changes (+) were seen in the radiograph, but autopsy showed n evidence of pulmonary oedema. Bronchiolitis did not occur (b) 10 rabbits which had been anuric for 48 hours were gi en an average of 1 2 (1.0—1 6) ml of N rkotal per kg body weight and Ringer solution. In 3 of them slight pulmonary oedema developed (as well as radiographic changes) nd bronchiolitis occurred m 7

These experimental conditions were not suitable as a higher frequency of pulmonary changes during an observation period shorter than that used by Alwall et al. was considered desirable. The 'Narkotal' dose was therefore increased to 1 7 ml per kg body weight. Further increase was not tried as it was found that highly uraemic animals did not tolerate the last mentioned dose (Table 18)

At a fixed induction time and dose of Narkotal highly uraemic animals became very feeble and died during injection at a rate that affected slightly uraemic animals very little. A fairly constant depth of sleep was therefore tried, which, when the dose was the same, meant that the duration of narcosis varied individually In highly uraemic animals reactions to pain and reflexes gave unsatisfactory guidance as regards the depth of narcosis. The animals could still show reflexes and react to pain after marked bradypnoea or even apnoea had set in. Therefore, the respiratory rate (abdominal respiration) was used as an indicator of the depth of narcosis.

However with this method, too it was found very difficult to control the depth of narcosis as exactly as was desired particularly in the highly uraemic animals. The difference between the amount of anaesthetic that produced a respiratory rate of 16 per minute and that which caused prolonged respiratory standstill was in many cases very small. The respiratory rate did not fall below 20 per minute in 19 % of the cases. In 23 % of the animals artificial respiration with a *rocking* device was necessary because of prolonged respiratory standstill lasting for 10 (2—50) *minutes* (Table 18) As a rule, a smaller amount of 'Narkotal was required to produce the desired bradypnoea in highly uraemic rabbits than in those with slight uraemia. Respiratory standstill was more difficult to avoid in highly uraemic ani

1 1/2 hours. The anuric animals were given penicillin and streptomycin prophylacti cally against infectious complications dur ing the uraemic stage. Pulmonary changes designated as fluid lung" or fluid reten tion lung" (Alwall 1963) appeared in 8 % of the animals that were operated upon under Narkotal narcosis. In those which after operation under Narkotal narcosis were given Ringer s solution in amounts equalling 15 % of the body-weight, radiographic pulmonary changes occurred in one-third within the first 24 hours and in two-thirds during the observation period. In 31 rabbits (weighing 1 11—2 49 kg) whose ureters were tied under Narkotal narcosis, the lung weight at bloodletting 48 hours later averaged 4 19 (3 40—9.86) g per kg body weight In 36 rabbits (weigh ing 1 79—2 48 kg) in which ureteral liga tion was made under Narkotal narcosis and infusions were given as above, the average lung weight 48 hours later was 4 9 (3 6—6 1) g per kg body weight.

In one experimental series (Lunder quist) the administered amount of fluid was removed by ultrafiltration of the blood in vivo about 20 hours after the operation under Narkotal narcosis and infusion of Ringer s solution the radiographic pulmonary changes and pleural effusion that had already appeared dis appeared in response to the ultrafiltration treatment and no radiographic pulmonary changes developed subsequently despite increasing uraemia.

The lung weight rose in the animals that exhibited radiographic changes Berg man summarizes the result of the microscopical examination of lungs with radiographic changes as follows — — — was noted pulmonary oedema of varying degree with a protein concentration in the oedematic fluid sufficiently high to be demonstrated by the histological technique used Inflammatory changes were not noted nor any fibrin deposit of the type described by Hopps Wissler as an autopsy finding in uraemic patients. This report also includes an experimental series in which amounts of Ringer s solution equalling 15 % of the animals body weights were infused 2 days after ureteral ligation under local anaesthesia. Under these ex perimental conditions, overhydration, which was induced in the presence of uraemia did not cause any radiographic pul monary changes during the observation period, that is, before the animals died in uraemia

Alwall et al. designated the noted radiographic changes in the experimental ani mals as "Narkotal uraemia fluid-retention lung concluding that overhydration is an essential pathogenetic factor as it is in uraemia fluid retention lung in patients (13, 16, 18, 20 21)

Own investigations

This series was used for studying the frequency of pulmonary oedema fol lowing Narkotal narcosis at varying degrees of uraemia in non-overhydrat ed and overhydrated rabbits whose ureters had been tied bilaterally

2 24 48 or 72 hours after their ure ters had been tied bilaterally the ani mals were anaesthetized with Nar kotal 1 7 ml per kg body weight The following experiment had to be carried out before the technique suitable for the applied conditions could be estab lished

The rabbits were placed on their back with their heads hanging about 2 cm low er than their hind paws. Narkotal was

Kindly placed at our disposal by AB A tra Sweden.

Narkotal ® solution contains iso-propyl beta bromallyl N malonylcarbamide sodi um, 0 055 g phenazone, 0 08 g glycerine 0 05 g, aqua sterilisata ad 1 ml.

Table 18 *Cardiac enlargement and pulmonary hanges seen in the radiographs before Narkotal anaesthesia and after Narkotal anaesthesia and infusion of Ringer s solution, 15 % f the body-weight for 1 ½ hours, in rabbits whose uret rs had been tied 2, 24 48 and 72 hours, respectively bef re the anaesthesia. The figures denote the number f rabbit in each group*

			Duration of anuria Number of animals 2 hours	24 hours	48 hours	72 hours	Total
Cardiac enlargement	Before anaesthesia	0	22	9	21	21	74
		+	—	1	3	2	6
		++	—	—	—	1	1
		+++	—	—	—	—	—
		N X-ray	—	—	—	2	2
	After infusion	0	11	6	11	14	42
		+	11	4	9	8	32
		++	1	—	3	3	7
		+++	—	—	—	—	—
		N X-ray	—	—	1	1	2
Pulmonary changes	Before anaesthesia	0	23	10	22	23	78
		+	—	—	2	1	3
		++	—	—	—	—	—
		+++	—	—	—	—	—
		N X-ray	—	—	—	2	2
	After infusion	0	22	9	19	18	68
		+	1	1	3	5	10
		++	—	—	1	2	3
		+++	—	—	—	—	—
		No X ray	—	—	1	1	2
		Total	23	10	24	26	83

were subjected to autopsy atelectasis wa noted but there was no pulmonary oedema Microscopical examination was performed in 3 cases no bronchi olitis w s seen.

Appearance and behaviour after na cosi The rabbits were sluggish and weak for several hours. Most of them then ecovered seemingly completely a few deteriorated further nd a few remained weak up to th time of blood letting

The respiratory rate which before the narcosis averaged 120 (38—108) per minute was 1 hour after the nar cosis 71 (20—144) per minute.

E.C.G and heart rate The heart rate before the narcosis averaged 223 (120—300) and 1 hour after the nar cosis 240 (182—327) beats per minute.

The electrocardiograms were un changed after the narcosis in compari son with those taken before the nar cosis in all the animals that had had anuria for 2 24 or 48 hours before the narcosis. Among 12 animals with an uria for 72 hours before the narcosis, the E.C.G was normal in 11 and the

Table 18 *Length of survival time of death and duration of 'Narkotal' anaesthesia and lowest respiratory rate during anaesthesia in those animals that survived the anaesthesia*

Time of death	Duration of anuria 2 hours	24 hours	48 hours	72 hours	Total
during anaesthesia before infusion	3	2	11	19	35
during infusion	1	0	5	0	6
spontaneous death within 24 hours of infusion	1	0	10	12	23
bled 24 hours after infusion	32	20	26	28	106
Number of anaesthetized rabbits	37	22	52	50	170
Duration of anaesthesia for rabbits that survived the anaesthesia	31	27	40	52	39
	(14—60)	(10—65)	(12—120)	(15—125)	(10—125)
Lowest respiratory rate during anaesthesia					
20 per minute	4	7	0	8	25
4—18 per minute	19	9	29	22	79
respiratory standstill (artificial respiration)	11	4	0	10	31

mals and was in them more often followed by cardiac arrest in spite of artificial respiration. Animals that became dyspnoeic and cyanotic tolerated only very slow injection of Narkotal solution The duration of narcosis in rabbits which needed artificial respiration averaged 54 (16—125) minutes and for those in which a satisfactory depth of narcosis was not achieved it lasted 27 (10—75) minutes. Highly uraemic animals took a long time to recover from the narcosis

At least half the animals in each series were immediately after the completion of the injection of Narkotal given Ringer s solution by intravenous infusion 15 % of the body weight for 1 1/2 hours, lying in the supine position during the procedure Those that after the termination of the experiment survived 24 hours were bled Four moribund animals with severe changes were bled within 22 hours of the experiment Respiratory rate measurements electrocardiograms, and chest films were taken before ligation of the ureters before induction of narcosis in rabbits with anuria for at least 24 hours, within 1 hour of the experiment and immediately before the bloodletting

Non-overhydrated animals
Reaction to Narkotal narcosis

Deaths during Narkotal narcosis Among 37 animals with anuria for 2 hours 8 % died, among 2? animals with anuria for 24 hours 0 died among 62 animals anuric for 48 hours 21 % died and among 50 animal with anuria for 72 hours 3? % died during the narcosis (Tabl 18) 20 animals

1 had no pulmonary changes. Dyspnoea was difficult to detect in animals with severe uraemia and a poor general condition.

The respiratory rate in 20 rabbits with pulmonary oedema immediately before the bloodletting averaged 135 (80—190) per minute. Among 25 animals which at the bloodletting had respiratory rates exceeding 120 per minute, 9 had pulmonary oedema, 2 had profuse pleural effusion, 7 had other slight pulmonary changes, and 7 had no or insignificant pulmonary changes.

E.C.G and heart rate E.C.G after the infusion showed no abnormalities in 58 animals, absent T waves in 14 negative T waves in 8 and marked deformation of the QRS-complex in 1 Among the 23 rabbits with E.C.G abnormalities after infusion, pulmonary oedema developed later in 14 Nineteen rabbits with pulmonary oedema had immediately before the bloodletting a mean heart rat of 230 (182—316) beats per minute E.C.C howed no abnormalities in 12 cases, absent T waves in 3 nd negati e T waves in 4 Serum pota sium wa determined at the bloodletting in 16 highly uraemic nimals. Among 11 with serum potassium exceeding 7.3 mEq per litre, the E.C.C showed no abnormalities in bsent T waves in 3, negative T wa es in 3, and a deformed QRS-complex in 1 Three rabbit with serum potassium levels of 7.4 7.7 and 10.2 mEq per litre respectively had pulmonary oedema of moderate degree

The heart shadow was enlarged in overhydrated rabbit only 15 out of 18 overhydrated rabbits with at least three criteria of pulmonary oedema had cardiac enlargement. 15 out of 41 rabbits with cardiac enlargement had at least three criteria of pulmonary oedema Among rabbits with amounts of ascitic fluid exceeding 180 ml, 23 out of 25 did not have cardiac enlargement A calculation of the correlation between the amount of fluid in the pericardial sac and the heart size in 86 overhydrated rabbits showed r = $= 0.31 \pm 0.10$ The method of measuring the pericardial fluid was not satisfactory however Electrocardiographic changes before the bloodletting were present predominantly in rabbits with severe uraemia. The correlation between the heart size and the severity of electrocardiographic changes was poor $r = 0.07 \pm 0.10$ as measured in 82 overhydrated rabbits an aesthetized with 'Narkotal'

Cardiac enlargement and slight pulmonary changes were present before the narcosis in 1 rabbit The animal was bled 24 hours later and was found to have pulmonary oedema. Before the narcosis 8 animals had cardiac enlargement pulmonary oedema developed in 6 animals.

Following overhydration, 11 rabbits had cardiac enlargement and pulmonary changes lung oedema developed in 82 %. 22 rabbits had after the infusion cardiac enlargement without pulmonary changes lung oedema developed in 23 %. 45 rabbits had normal heart-size and no pulmonary changes after the infusion lung oedema arose in 20 %

Radiographic pulmonary changes

Lindered

T wave was absent in 1 One hour after the narcosis these 12 had normal electrocardiograms the T wave was absent in 2 and negative in 4 and the QRS complex markedly deformed in 2 In most cases the E C G abnormalities persisted or became more severe during the first 24 hours following the narcosis

The heart shadow before the narcosis was enlarged in 1 rabbit Radiographic pulmonary changes were not noted. One hour after the narcosis chest X ray in the non infusion group (46 animals) showed no abnormalities.

Overhydrated animals
Reaction to Narkotal' narcosis and overhydration

Deaths during infusion of Ringer s solution 6 out of 89 animals died during the infusion Autopsy was performed in 4 cases and revealed bluish red sanguineous lungs but no frothy fluid in the air ways In 1 case the lungs were pink histological examination showed only mild atelectasis.

As regards the appearance and behaviour the animals did not differ much from the non-overhydrated series

The respiratory rate before the narcosis averaged 125 (58—200) and after narcosis and infusion 75 (32—132) per minute.

E.C.G and heart rate The heart rate before the narcosis averaged 224 (152—300) and after narcosis and infusion 240 (125—308) beats per minute.

The electrocardiograms were normal both before narcosis and after infusion in all the animals in the groups with narcosis plus infusion 2 and 24 hours after ureteral ligation In the group of 24 rabbits with narcosis plus infusion 48 hours after ureteral ligation the E.C G showed no abnormalities in 20 isoelectric T waves in 2 and negative T waves in 2 after narcosis and infusion the E C.G was normal in 15 the T wave isoelectric in 3 and negative in 3 and the QRS-complex was deformed in 1 (the record is missing in 1 case) In the group of 26 rabbits with narcosis plus infusion 72 hours after ureteral ligation the E.C G before narcosis showed no abnormalities in 21 isoelectric T waves in 2 and negative T waves in 2 (the record is missing in 1) After narcosis and infusion the E.C.G. was normal in 10 the T wave isoelectric in 10 and negative in 6

The heart size before the narcosis was increased in 9 % of the rabbits, as against 48 % after narcosis and infusion (Table 19)

Pulmonary changes were noted before the narcosis in 4 % and after the infusion in 16 % of the animals, predominantly in those with high degrees of uraemia (Table 19)

Summary of the findings in non-overhydrated and overhydrated rabbits with at least three criteria of pulmonary oedema following Narkotal' narcosis

Appearance and behaviour Severe dyspnoea was present in 10 animals 13 of these had pulmonary oedema 2 had profuse pleural effusion 3 had bronchitis or marked bronchiolitis and

non-overhydrated animals. In the non overhydrated group the highest value was 85.8 % one overhydrated rabbit had a water content of 91 1 % which was the highest value recorded for all the series. In spite of very high water contents, even those amounting to 91 1 % and 90 7 % the alveolar oedema was clearly visible microscopically. Most of the animals with more than 200 ml of ascitic fluid had a low water content in the lungs.

Microscopical examination showed alveolar oedema in 30 animals. The preparation was accidentally lost in 1 case no examination was performed in 27 cases, as the macroscopical pictures and the lung weights were normal (Tables 20 and 21) Alveolar oedema was seen in all except 3 of the 29 rabbits that had pulmonary oedema evidenced by at least three criteria. Among the 3 exceptions, 1 had very large haemorrhages, 1 had hyperaemia and 1 had only slight changes. In 4 cases alveolar oedema was noted but pulmonary oedema according to three criteria could not be established. The al eolar oedema was clearly visible in 18 cases in 12 cases it was only seen focally or indistinctly Most of the rabbits had alveolar oedema with fibrinous stringy exudate, others had haemorrhagic al eolar oedema with protein-rich exudate, and 1 rabbit had thin vacuolized oedematic fluid. In some animal all the different types were seen and the degree of visibility and stringiness varied from one part of the lung t another

Criteria of pulmonary oedema

The six criteria of pulmonary oedema used here showed relatively good correlation in this series In 29 cases the diagnosis of pulmonary oedema was established and in 34 % it was supported by all the six criteria in 34 % it was supported by five in 21 % by four and in 10 % by three criteria Radiographs were missing in 5 cases and the microscopical preparation was lost in 1 case.

In 91 cases the number of positive criteria was less than three and pulmonary oedema was therefore considered not present. 65 of these rabbits had no positive criteria 16 had one criterion, and 10 had two. Radiographs are missing in 2 cases. In 26 cases the lungs were not examined microscopically as the gross pictures and the lung weights were normal and there was no frothy fluid. None of these rabbits had more than one positive criterion of pulmonary oedema.

"Pulmonary oedema following Narkotal" narcosis in uraemic rabbits (Diagram 18 Tables 20 and 21) was characterized by distinct gross pulmonary changes, in most cases without haemorrhages and usually clearly visible alveolar oedema. In the majority of cases X ray and measurements of lung weight to body weight ratio provided clear evidence. Observations on the water content and frothy fluid, on the other hand, were less valuable in estimating the degree of the pulmonary oedema that arose under the experimental conditions of this study

A characteristic feature of the lung oedema in this series was that, when

were present in 31 out of 120 animals 7 spontaneously dead animals were *not examined. Films of those dying* spontaneously were taken only if there *was time to do so during agony* The X ray findings were in most cases consistent with other findings Radiographic changes were present in 10 rabbits without pulmonary oedema by *at least three criteria* 6 of these had atelectasis and profuse pleural effusion 1 had mild pneumonia and 3 had moderate lung bleedings whether they also had hyperaemia is uncertain. 3 rabbits had at least three criteria of pulmonary oedema without radiographic changes In 2 of these the *oedema was located basally and in 1* it appeared here and there in the lungs

Gross observation at autopsy 39 rabbits had normal lung surfaces without changes most of them being slightly uraemic non-overhydrated rabbits. *A few* red or brown dots and spots were seen on the lung surfaces of 32 rabbits most of which were highly uraemic. Microscopical examination showed focal haemorrhages. *13 over* hydrated rabbits with in most cases profuse pleural effusion had bluish red atelectatic lungs. In several rabbits in the two last mentioned groups numerous white dots were seen on the lung surfaces

Gross evidence of pulmonary oedema was present in 36 animals. 13 of these had slight generalized *changes in* the form of numerous red dots or slightly *diffuse* spotty markings and/or localized changes in the form of reddish brown, in most cases numerous, spots basally or in the hilar region, or spotty markings in a small part of the lung 11 rabbits had moderate macroscopical changes in the form of numerous *large irregular spots*, innumerable small red dots, or mottled lung surfaces *seen only in the lower lobes* or parts thereof In the 12 rabbits with the most extensive macroscopical changes the lungs had mottled surfaces of varying colour-combinations, *for* instance, purple—beige blue—brown red—brown—white red—pink. In 29 out of the above-said 36 rabbits the macroscopical diagnosis of pulmonary oedema was further supported by at least two other criteria. All the *rabbits with pulmonary oedema* by at least three criteria had also gross evidence *of pulmonary* oedema (Diagram *18)*

Frothy fluid in the air ways was found in 23 animals 1 of these had bronchitis and the rest had pulmonary oedema 7 animals with at least three criteria of pulmonary oedema had no frothy fluid in the air ways

The lung weight was increased in *25 animals In 4 cases* it was within normal range although at least three other criteria of pulmonary oedema were present (Tables 20 and 21) In these cases the changes were localized in a small part of the lung

The water content of the lung was increased in 34 animals. 7 animals with pulmonary oedema had normal water *contents 12 without three criteria* of pulmonary oedema had increased water contents (Tables 20 and 21) The average water content of the lung was much higher in overhydrated than in

non-overhydrated animals. In the non overhydrated group the highest value was 83.8 % one overhydrated rabbit had a water content of 91 1 % which was the highest value recorded for all the series. In spite of very high water contents, even those amounting to 91 1 % and 90.7 % the alveolar oedema was clearly visible microscopically Most of the animals with more than 200 ml of ascitic fluid had a low water content in the lungs.

Microscopical examination showed alveolar oedema in 50 animals. The preparation was accidentally lost in 1 case: no examination was performed in 27 cases, as the macroscopical pictures and the lung weights were normal (Tables 20 and 21) Alveolar oedema was seen in all except 3 of the 20 rabbits that had pulmonary oedema evidenced by at least three criteria Among the 3 exceptions, 1 had very large haemorrhages, 1 had hyperaemia, and 1 had only slight changes. In 4 cases alveolar oedema was noted but pulmonary oedema, according to three criteria could not be established. The alveolar oedema was clearly visible in 18 cases in 12 cases it was only seen focally or indistinctly Most of the rabbits had alveolar oedema with fibrinous stringy exudate others had haemorrhagic alveolar oedema with protein-rich exudate, and 1 rabbit had thin vacuolized oedematic fluid. In some animals all the different types were seen and the degree of visibility and stringiness varied from one part of the lung to another

Criteria of pulmonary oedema

The six criteria of pulmonary oedema used here showed relatively good correlation in this series In 29 cases the diagnosis of pulmonary oedema was established and in 34 % it was supported by all the six criteria In 34 % it was supported by five, in 21 % by four and in 10 % by three criteria. Radiographs were missing in 5 cases and the microscopical preparation was lost in 1 case

In 91 cases the number of positive criteria was less than three and pulmonary oedema was therefore considered not present 65 of these rabbits had no positive criteria 15 had one criterion and 10 had two Radiographs are missing in 2 cases. In 26 cases the lungs were not examined microscopically as the gross pictures and the lung weights were normal and there was no frothy fluid. None of these rabbits had more than one positive criterion of pulmonary oedema.

"Pulmonary oedema following Narkotal narcosis in uraemic rabbits (Diagram 18, Tables 20 and 21) was characterized by distinct gross pulmonary changes, in most cases without haemorrhages and usually clearly visible alveolar oedema In the majority of cases X ray and measurements of lung weight to body weight ratio provided clear evidence Observations on the water content and frothy fluid, on the other hand, were less valuable in estimating the degree of the pulmonary oedema that arose under the experimental conditions of this study

A characteristic feature of the lung oedema in this series was that, when

were present in 31 out of 120 animals 7 spontaneously dead animals were not examined. Films of those dying spontaneously were taken only if there was time to do so during agony The X ray findings were in most cases consistent with other findings Radiographic changes were present in 10 rabbits without pulmonary oedema by at least three criteria 6 of these had atelectasis and profuse pleural effusion 1 had mild pneumonia and 8 had moderate lung bleedings whether they also had hyperaemia is uncertain 3 rabbits had at least three criteria of pulmonary oedema without radiographic changes In 2 of these the oedema was located basally and in 1 it appeared here and there in the lungs

Gross observation at autopsy 39 rabbits had normal lung surfaces without changes most of them being slightly uraemic non overhydrated rabbits A few red or brown dots and spots were seen on the lung surfaces of 32 rabbits, most of which were highly uraemic. Microscopical examination showed focal haemorrhages. 13 overhydrated rabbits with in most cases profuse pleural effusion had bluish red atelectatic lungs. In several rabbits in the two last mentioned groups numerous white dots were seen on the lung surfaces.

Gross evidence of pulmonary oedema was present in 30 animals 13 of these had slight generalized changes in the form of numerous red dots or slightly diffuse spotty markings, and/or localized changes in the form of reddish brown, in most cases numerous, spots basally or in the hilar region or spotty markings in a small part of the lung 11 rabbits had moderate macroscopical changes in the form of numerous large irregular spots, innumerable small red dots or mottled lung surfaces seen only in the lower lobes or parts thereof In the 12 rabbits with the most extensive macroscopical changes the lungs had mottled surfaces of varying colour combinations, for instance purple—beige blue—brown red—brown—white red—pink. In 29 out of the above said 30 rabbits the macroscopical diagnosis of pulmonary oedema was further supported by at least two other criteria All the rabbits with pulmonary oedema by at least three criteria had also gross evidence of pulmonary oedema (Diagram 18)

Frothy fluid in the air ways was found in 23 animals 1 of these had bronchitis and the rest had pulmonary oedema 7 animals with at least three criteria of pulmonary oedema had no frothy fluid in the air ways

The lung weight was increased in 25 animals. In 4 cases it was within normal range although at least three other criteria of pulmonary oedema were present (Tables 20 and 21) In these cases the changes were localized in a small part of the lung

The water content of the lung was increased in 34 animals. 7 animals with pulmonary oedema had normal water contents 12 without three criteria of pulmonary oedema had increased water contents (Tables 20 and 21) The average water content of the lung was much higher in overhydrated than in

III. Anaesthesia 48 hours after ligation of ureters	K L	6.28	83.8	++	+	+++	0	●	+	3	1	228	25	
	L	5.83	84.2	+++	+	++	●	0	+	1	1	230	25	
		4.01	81.7	●	0	●	●	0	(+)	0	63	221	26	
		4.07	80.6	0	0	—	●	0	—	0	183	300	23	
		3.97	80.0	0	●	—	●	0	—	5	65	265	23	
		3.85	79.8	●	0	0	●	0	+	0	0	240	25	
		3.90	82.6	●	●	—	●	0	—	1	4	263	25	
		3.67	81.8	0	●	0	●	0	0	2	100	246	25	
		3.06	81.3	0	0	—	0	0	—	0	190	262	26	
		3.13	79.6	●	0	—	●	0	—	0	9	231	25	
										1	67	208		
Mean		4.36	81.0											
IV Anaesthesia 72 hours after ligation of ureters	L	5.37	84.9	++	++	+	+	0	+	13	75	261	21	
	K L	5.52	81.3	+	0	0	+	●	+	0	88	365	22	5
		4.33	80.8	0	●	0	0	0	0	1	70	308	12	
	K	5.04	81.8	0	●	●	0	0	+	0	163	313	24	
	K	5.06	79.5	●	0	●	0	0	(+)	3	210	288	13	
		4.87	80.0	0	0	—	●	●	—	8	178	311	1	5
		4.76	81.1	0	0	0	0	0	(+)	1	201	400	24	
	K	4.73	80.3	●	0	0	0	0	+	0	107	316	24	
		4.60	81.7	●	●	0	0	0	+	3	31	350	24	
	K	4.43	80.7	0	●	0	0	0	(+)	0	6	300	4	
		4.21	81.2	●	0	0	0	0	+	0	30	310	21	
		3.96	78.5	0	●	0	0	0	(+)	6	147	258	21	
										2	110	331		
Mean		4.91	81.0											

Table 20 *Results after 'Narkotal' anaesthesia in anuric overhydrated rabbits whose ureters had been tied 2 24 48 and 72 hours respectively before the anaesthesia K=artificial respiration L=at least three criteria of lung oedema S=spontaneous death*

		Lung weight g/kg body weight	Water content in lung %	Pulmonary oedem by gross observation	Frothy fluid in th al -w ys	Alveolar oedema	X ray Lung cha ges	X ray Cardiac enlargement	Bronchiolitis	Pleural fluid ml	Ascites ml	N P N mg per 100 ml	Length of survival after anaesthesia hours
I. Anaesthesia 2 hours after ligation of ureters	K	5.13	81 7	0	0	0	0	0	(+)	0	0	183	20
		5 12	81 1	0	0	0	0	0	0	0	14	113	25
	K	4 16	82.1	0	0	—	0	0	—	0	0	148	26
		4.37	83 1	0	0	0	0	0	(+)	0	0	110	24
		4.22	79.3	0	0	0	0	0	0	0	03	150	21
		4 16	82 0	0	0	—	0	0	—	0	2	115	23
		3.88	81.3	0	0	—	0	0	—	0	0	138	24
		3.82	81.6	0	0	—	0	0	—	0	0	150	24
		3.75	80.2	0	0	—	0	0	—	0	0	137	25
		3.50	81.2	0	0	—	0	0	—	0	0	127	25
Mean		4.[illegible]5	81 1							[illegible]	8	130	
II Anaesthesia 24 hours after ligation of ureters		4.62	80.5	0	0	0	0	0	0	1	110	220	28
	K	4 00	81.3	0	0	0	0	0	0	0	3	151	26
		4.37	82 6	0	0	+	+	0	+	0	1	210	25
		4.23	82.1	0	0	—	0	0	—	0	0	203	20
		4 70	80 7	0	0	—	0	0	—	0	0	183	20
	K	4 17	80 8	0	0	—	0	0	—	0	10	181	23
	K	4 12	82 1	0	0	0	0	0	(+)	1	10	185	20
		3 93	81 5	0	0	—	0	0	—	1	0	180	21
		3 91	80 0	0	0	—	0	0	—	0	57	108	26
		3 31	81 0	0	0	—	0	0	—	2	3	153	27
Mean		4 15	81 7							0.5	19	191	

II. Anaesthesia 24 hours after ligation of ureters		L	6.13	86.1	++	+	++	++	++	+	19	75	161	28	
		L	5.25	83.7	+	●	++	+	++	+	15	33	196	27	
		L	5.14	83.1	+	+	+	+	+	+	4	8	167	23	
			5.12	83.4	●	●	0	0	+	(+)	7	76	200	27	
			4.83	83.3	0	0	0	0	+++	●	11	80	151	25	
			4.74	81.0	+	0	0	0	++	0	13	63	228	23	
	K		4.50	84.3	●	0	+	0	+	+	24	32	168	23	
			4.48	83.8	●	●	●	0	++	●	12	85	164	25	
			4.37	83.8	0	0	●	0	0	+	10	280	140	29	
			3.96	82.8	●	0	●	0	0	0	22	17	167	28	
Mean			4.83	83.1							14	83	172		
III. Anaesthesia 48 hours after ligation of ureters		L	13.44	90.7	+++	++	+++	—	—	0	17	17	195	6	S
		L	14.41	91.1	+++	++	+++	—	—	0	15	37	—	3	S
		L	10.33	85.8	+++	++	+++	+++	?	+	27	24	203	26	
		L	9.06	86.7	+++	++	+++	+	+++	+	25	97	277	24	
		L	8.58	88.1	++	++	+	—	—	0	25	151	—	19	S
	K	L	8.36	86.0	++	0	+	—	—	0	24	22	306	3	S
	K	L	7.79	83.3	++	0	●	+	+	●	3	55	316	6	S
		L	7.78	85.1	++	+	—	—	—	—	5	23	—	4	S
	K	L	6.81	84.5	++	++	+++	++	+++	+	34	180	271	28	
		L	5.53	83.2	+++	++	++	++	+	+	34	286	190	25	
		L	5.31	82.9	+	0	+	+	++	0	7	20	178	28	
			5.11	82.7	+	0	●	+	0	+	26	237	188	27	
			4.77	82.6	0	0	+	+	+++	+	19	33	233	29	
			4.63	82.6	+	0	+	—	—	+	26	227	263	27	
			4.31	82.1	0	0	●	0	●	+	12	246	233	26	
			4.27	80.2	0	0	—	0	0	—	2	433	171	26	
			4.24	81.3	0	0	0	●	0	0	6	637	221	26	
			4.12	81.6	●	0	0	0	0	+	8	201	212	25	
			4.09	81.0	0	●	—	—	—	—	13	103	—	23	S
			3.81	83.9	0	●	●	++	+	0	12	292	242	23	
			3.79	82.8	0	●	—	●	0	—	10	363	301	29	
			3.77	83.3	0	●	—	0	+	—	8	138	159	28	
			3.78	83.3	0	●	0	0	0	0	3	560	179	29	
Mean			6.52	84.1							16	193	228		

Table 21 *Results after 'Narkotal' anaesthesia and infusion of Ringer's solution amounting to 15 % of the body-weight for 1 1/2 hours in anuric rabbits whose ureters had been tied 2, 24, 48 and 72 hours respectively before the anaesthesia. K=artificial respiration. L=at least three criteria of lung oedema. S=spontaneous death*

I Anaesthesia 2 hours aft ligation of uret rs		Lung weight g/kg body weight	Water content in lung %	Pulmonary oedema by gross observation	Frothy fluid in the air-ways	Alveola oedema	X ray: Lung changes	X ray: Cardiac enlargement	Bronchiolitis	Pleural fluid ml	Ascites ml	N P N. mg per 100 ml	Length of survival after anaesthesia hours
	K L	0.02	83.5	+	0	+	+	++	+	5	5	107	25
		5.18	82.2	0	0	0	0	+	0	3	2	158	25
		5.38	83.6	0	0	0	0	0	+	12	28	180	26
		5.31	85.8	++	0	0	0	++	0	7	20	87	26
	K	5.23	83.8	0	++	0	+	+++	+	12	7	117	25
		5.14	82.0	+	0	0	0	++	0	27	6	97	26
		5.03	82.5	0	0	0	0	+	0	0	30	98	26
		5.02	83.1	0	0	—	0	+	—	5	85	104	26
	K	4.01	83.3	0	0	0	0	++	0	7	10	110	25
	K	4.87	83.2	0	0	0	+	++	0	17	53	83	26
	K	4.85	83.8	0	0	0	0	++	+	7	3	174	25
		4.78	83.0	0	0	0	++	++	0	32	30	82	23
	K	4.73	82.4	0	0	0	+	+++	(+)	23	22	142	25
		4.07	81.3	0	0	0	0	0	0	8	12	107	25
		4.03	83.1	0	0	0	0	++	0	34	33	81	26
		4.41	83.3	0	0	0	0	+++	0	31	63	121	23
		4.42	83.3	0	0	—	0	+	—	7	4	98	20
	K	4.33	82.0	0	0	0	0	+++	0	20	25	152	25
		4.22	82.5	0	0	0	++	+++	(+)	41	52	121	[illegible]
		3.96	83.3	0	0	—	0	0	—	18	10	126	28
		3.83	82.7	0	0	0	0	+	0	44	31	92	25
		3.53	81.8	0	0	—	0	0	—	20	163	78	20
	Mean	4.77	83.2							18	32	112	

II. Anaesthesia 24 hours after ligation of ureters		L	6.12	80.1	++	+	++	++	++	+	19	76	161	36	
		L	5.28	80.7	+	●	++	+	++	+	18	35	190	27	
		L	5.11	83.1	+	+	+	+	+	+	4	8	167	35	
			5.17	83.1	0	●	0	●	+	(+)	7	76	200	27	
			5.03	85.3	0	0	●	●	+++	●	11	60	161	36	
			4.71	81.8	+	●	●	0	++	0	12	62	220	33	
	K		4.62	81.3	●	●	+	0	+	+	24	62	196	32	
			4.48	80.8	●	0	0	●	++	0	12	85	164	25	
			4.37	83.8	0	●	0	●	0	+	18	180	140	29	
			3.86	82.0	●	●	0	0	●	●	22	17	167	28	
Mean			4.81	84.1							14	85	172		
III. Anaesthesia 48 hours after ligation of ureters		L	15.41	90.7	+++	++	+++	—	—	0	17	17	106	6	9
		L	14.41	91.1	+++	++	+++	—	—	●	15	37	—	3	8
		L	10.28	83.8	+++	++	+++	+++	?	+	22	24	202	26	
		J	9.06	80.7	+++	++	+++	+	+++	+	33	97	277	21	
		L	8.84	83.1	++	++	+	—	—	0	35	161	—	10	9
	K	I	8.36	86.0	++	●	+	—	—	0	24	73	308	3	5
	λ	L	7.79	85.3	++	0	0	+	+	●	5	65	216	8	5
		L	7.78	80.1	++	+	—	—	—	—	6	73	—	1	5
	K	L	6.81	81.8	++	++	+++	++	+++	+	34	180	371	36	
		L	6.03	82.2	+++	++	++	++	+	+	21	445	180	28	
		L	5.21	83.9	+	0	+	+	++	●	7	76	178	48	
			5.11	82.7	+	0	0	+	0	+	26	267	168	37	
			4.77	82.6	●	●	+	+	+++	+	10	83	235	29	
			4.65	82.6	+	●	+	—	—	+	48	337	280	37	
			4.31	82.1	0	0	0	0	●	+	12	356	253	20	
			4.27	80.2	0	0	—	●	●	—	3	435	171	26	
			4.21	81.3	0	0	0	●	0	●	8	437	221	34	
			4.12	81.9	●	0	0	●	0	+	0	201	212	33	
			4.08	81.8	0	0	—	—	—	—	15	107	—	32	4
			3.81	83.9	●	0	0	++	+	0	12	292	212	25	
			3.79	82.8	●	0	—	0	●	—	10	303	301	29	
			3.77	83.3	0	0	—	0	+	—	8	138	169	28	
			3.76	83.3	0	0	0	0	0	0	3	500	179	29	
Mean			6.52	81.1							10	188	225		

Table 21 *Results after Narkotal anaesthesia and infusion of Ringer's solution amounting to 15 % of the body-weight for 1 1/2 hours in anuric rabbits whose ureters had been tied 2 24 48 and 72 hours respectively before the anaesthesia k = artificial respiration. L = at least three criteria of lung oedema. S = spontaneous death*

			Lung weight g/kg body weight	Water content l lu g %	Pulmonary oedema by gross bservation	Frothy fluid in the air way	Alveolar oedema	X ray: Lung changes	X ray: Cardiac engorgement	Bronchiolitis	Pleural fluid ml	Ascites ml	N P N mg per 100 ml	Length of survival after anaesthesia hours	
B An[a]esthesia 72 hours after ligation of ureters		L	13.06	88.0	+++	++	+++	+++	?	+	4	31	241	8	S
	k	L	11.97	84.6	+++	+++	0	+++	++	0	15	175	315	4	S
		L	11 79	87.2	+++	++	++	++	+	+	4	62	346	0	S
		L	10 [illegible]0	89.0	+++	+++	+	+	+	0	7	48	178	3	S
		L	9 00	89 1	+++	+++	++	++	+	0	10	40	230	2	S
		L	7 06	84.2	++	++	+++	+	0	+	17	236	265	25	
		L	7 47	82.5	++	+	+++	+	++	0	12	165	281	25	
		I	7 19	81.8	+	+	++	+	0	+	7	176	359	25	
		L	6 48	82.8	+++	0	+++	+	++	0	31	162	277	25	
		L	6.27	80 8	+	+	++	0	0	+	2	378	287	23	
	k		1.81	82.1	+	0	0	0	++	0	8	78	205	23	
			4 79	82.3	0	0	0	0	0	+	3	322	310	25	
			1 02	80 7	0	0	0	0	0	0	2	342	301	22	
			1 57	80 6	0	0	0	0	0	0	1	376	314	25	
	K		1.20	81 6	+	0	0	0	0	+	10	202	200	26	
			1.37	80 8	0	0	0	0	0	0	0	370	303	25	
			4.30	81 9	0	0	0	++	0	+	51	155	302	25	
			4 33	82.6	0	0	—	0	0	—	14	251	280	27	
	K		1.08	81 1	0	0	0	0	+	0	12	1[illegible]0	301	28	
			1.01	80.0	0	0	0	0	0	0	7	113	300	23	
			4 02	81 0	0	0	0	0	0	+	1	22	332	25	
			1.82	81 0	0	0	0	0	0	0	16	283	238	26	
			3 77	80 9	0	0	0	0	0	0	10	381	227	25	
Mean			0 13	83 0							11	211	287		

slight, it occurred in patches, over the hilar or basal regions. In these cases the macroscopical and microscopical pictures established the diagnosis. In 3 there were no radiographic pulmonary changes, in 4 the lung weights were normal and in 5 the water contents were normal. Atelectasis, hyperaemia haemorrhages into the lung parenchyma and pneumonia were present though in a low frequency and did not cause any very great diagnostic difficulties.

Pulmonary oedema and uraemia

It will be seen from Tables 20 and 21 and from Diagrams 8 and 9 that the frequency of pulmonary oedema as defined here was as follows

A Non-overhydrated animals

Narkotal narcosis 2 hours after ureteral ligation The series comprised 10 animals which were bled within 24 hours of the narcosis. Pulmonary oedema was not noted in any of the cases.

Narkotal narcosis 24 hours after ureteral ligation 10 rabbits were bled within 24 hours of the narcosis. Pulmonary oedema was not noted in any of the cases.

Narkotal narcosis 48 hours after ureteral ligation The series comprised 10 rabbits which were bled within 24 hours of the narcosis. Pulmonary oedema wa noted in 2 cases.

Originally the series included f rther 2 animals, but they died spontaneously bef re the bloodletting, and as thei bodies were already cold and rigid when dis covered, the presence of pulmonary oedema could not be ascertained.

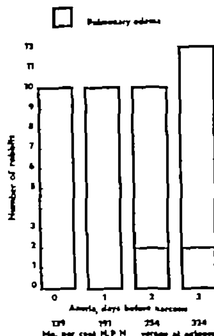


Diagram 8 Occurrence of pulmonary edema after 'Narkotal' anaesthesia in non-overhydrated rabbits which had been anuric for 0, 1 2, and 3 days, respectively before the induction of anaesthesia.

Narkotal narcosis 72 hours after ureteral ligation The series comprised 12 animals 9 were bled within 24 hours and 1 which was moribund, within 12 hours of the narcosis 2 died spontaneously before the bloodletting Pulmonary oedema was noted in 2 cases.

The series included originally a further 2 animals which died spontaneously nd were already cold and rigid t autopsy pulmonary oedema wa probably not present.

Table 21 *Results after "Varkotal" anaesthesia and infusion of Ringer's solution amounting to 15 % of the body weight for 1 1/2 hours in anuric rabbits whose ureters had been tied 2, 24, 48 and 72 hours respectively before the anaesthesia k = artificial respiration. L = at least three criteria of lung oedema. S = spontaneous death*

IV Anaesthesi 72 hours afte ligation of uret rs

		Lung weight g/kg body weight	W te content i lung %	Pulmonary oedema by gross bserv tion	Frothy fluid i the i ways	Al eolar oedema	X ray: Lung hangea	X ray: Cardia engarge-ment	Bronchio-l litis	Pleural fl id ml	Ascites ml	N P N mg per 100 ml	Length of survi l after naesthesia h rs	
	L	13 96	88 6	+++	++	+++	+++	?	+	4	31	241	8	S
K	L	11.07	81.6	+++	+++	0	+++	++	0	15	175	315	4	S
	L	11 79	87.2	+++	++	++	++	+	+	4	62	315	0	S
	L	10 79	80 0	+++	+++	+	+	+	0	7	48	178	3	S
	L	9 09	80 1	+++	+++	++	++	+	0	10	40	230	2	S
	L	7.96	81.2	++	++	+++	+	0	+	17	230	205	25	
	L	7 47	82.5	++	+	+++	+	++	0	12	165	281	25	
	L	7 19	81.8	+	+	++	+	0	+	7	175	350	25	
	L	0 18	82.8	+++	0	+++	+	++	0	31	162	277	25	
	L	5 27	80.8	+	+	++	0	0	+	2	370	287	23	
k		1.81	82.1	+	0	0	0	++	0	8	78	205	23	
		1 79	82.3	0	0	0	0	0	+	3	322	310	25	
		4 02	80 7	0	0	0	0	0	0	2	342	301	22	
		4 57	80 0	0	0	0	0	0	0	1	375	341	25	
k		1.30	81.6	+	0	0	0	0	+	10	202	200	26	
		1 37	80.8	0	0	0	0	0	0	0	370	303	25	
		4.36	81.9	0	0	0	++	0	+	54	155	302	25	
		4.33	82 6	0	0	—	0	0	—	14	254	280	27	
K		1 08	81 1	0	0	0	0	+	0	12	176	301	28	
		4.01	80 0	0	0	0	0	0	0	7	413	300	23	
		4 02	81.9	0	0	0	0	0	+	1	22	332	25	
		3 82	81.0	0	0	0	0	0	0	16	283	238	70	
		3 7	80 9	0	0	0	0	0	0	10	381	227	23	
Mean		6 13	83 0							11	211	287		

mic (at least 48 hours of anuria) over hydrated rabbits, in 18 % of the severely uraemic non-overhydrated rabbits, in 13 % of the slightly (anuria for less than 48 hours) uraemic overhy drated rabbits, and in none of the slightly uraemic non-overhydrated rabbits. The non-overhydrated rabbits and those with uraemia of low degree had only slight pulmonary oedema.

The frequency of pulmonary oedema following 'Narkotal' narcosis and overhydration was higher in severely (at least 48 hours of anuria) than in slightly (anuria for less than 24 hours) uraemic rabbits. The difference is significant.

7 % of the animals died during the night, at times when they were not under observation, and as autopsy was not performed until their bodies had become cold and rigid, the presence of pulmonary oedema could not be ascertained. This fact does not seem to have influenced the results of the investigation, since it was exactly the highly uraemic animals that died, and since the difference in numbers between overhydrated and non-over hydrated nimals was very small.

Pulm nary oedema and overhydration

Pulmonary oedema following Narkotal narcosis occurred more frequent ly among overhydrated than among non-overhydrated uraemic rabbits whose ureters had been ligated. This observation refers to all degrees of uraemia but the difference wa great est for severely uraemic rabbits ($p <$ 0 08)

Pulmonary oedema and depth and duration of narcosis

The frequency of pulmonary oedema as defined here has been calculated for 120 overhydrated or non-overhydrated rabbits which were anaesthetized with 'Narkotal' During the anaesthesia 29 of these rabbits had respiratory stand still which lasted long enough to necessitate artificial respiration pulmonary oedema developed in 31 % of this group During the anaesthesia 69 rabbits had a minimum respiratory rate of 4—18 per minute pulmonary oede ma developed in 17 % of these cases. In 22 rabbits the minimum respiratory rate was 20 per minute pulmonary oedema developed in 36 %

59 rabbits were anaesthetized with Narkotal for 30 minutes or less pul monary oedema developed in 24 % Among 61 rabbits anaesthetized for longer than 30 minutes, pulmonary oedema arose in 25 %

Accordingly these results lend no support to the assumption that the frequency of pulmonary oedema under the experimental conditions used here would be related to the depth and the duration of anaesthesia.

Microscopical findings notably bronchiolitis

Bleedings occurred in 5 % of the examined lungs, predominantly as focal small haemorrhages but also as diffuse occurrence of red cells in alve oli accompanied by an increase of alveolar phagocytes. Large haemor rhages occurred in rabbits with pul monary oedema, but only in a few

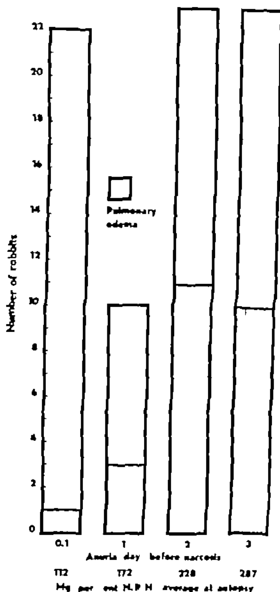


Diagram 9 Occurrence of pulmonary edema after 'Narkotal' anaesthesia and intravenous infusion of Ringer's solution, 15 % of the body weight for 1 /s hours, in rabbits which had been anuric f r 0 1 1 2, and 3 days, respectively before ind ction of anaesthesia.

B Overhydrated animals

Narkotal narcosis and overhydration 2 hours after ureteral ligation The series comprised 22 rabbits which were bled within 24 hours of the narcosis. Slight pulmonary oedema was noted in 1 case

Originally the series included 1 more rabbit which died spontaneously after the in fusion autopsy was performed too late for ascertainment of the presence of pulmonary oedema

Narkotal narcosis and overhydration 24 hours after ureteral ligation The series comprised 10 animals which were bled within 24 hours of the nar cosis Slight pulmonary oedema was noted in 3 cases.

Narkotal narcosis and overhydra tion 48 hours after ureteral ligation Among the 23 animals in the series, 16 were bled within 24 hours of the nar cosis and 7 died spontaneously before the bloodletting Pulmonary oedema was noted in 11 cases.

The series included 1 more rabbit which died spontaneously as the body was cold and rigid at autopsy the presence of pul monary oedema could not be ascertained

Narkotal narcosis and overhydration 72 hours after ureteral ligation The series comprised 23 animals 18 were bled within 24 hours of the narcosis, and 5 died spontaneously before the bloodletting Pulmonary oedema was noted in 10 cases.

The series included 3 more rabbits which died spontaneously as the bodies were cold and rigid at autopsy the presence of pulmonary oedema could not be ascer tained

Pulmonary oedema developed within 24 hours in 40 % of the severely urae

CHAPTER [illegible]

Pulmonary oedema following injection of adrenaline

Pulmonary oedema following injection of adrenaline in experimental animals was first described by Bouchard & Claude in 1902 and has later been reported by many other authors (e.g 78, 138, 183) Several surveys of the literature on the subject have been published (69 114 168 211)

Adrenaline produces lung oedema in rabbits, guinea-pigs, and mice. Dogs and cats die from suffocation or heart failure without pulmonary oedema (69) Pulmonary oedema follows injection administered intravenously (e.g. 167) intramuscularly (68) subcutaneously (33) intraperitoneally (209) or intratracheally (25) The individual variations are great. After a certain dose some animals survive without developing lung oedema, in some lung oedema follows, and others die without developing lung oedema (e.g. 167 183, 211 237) Dreckhahn (69) noted pulmonary oedema, diagnosed by increased lung weight, in half the rabbits 15—30 minutes after intramuscular injection of 3 mg *of* adrenaline per kg body weight the pressure in the pulmonary vein rose rapidly within 2 minutes. *Braun (42) without giving any other data* stated that adrenaline can cause pulmonary oedema even in nephrectomized rabbits.

The generation of pulmonary oedema after injection of adrenaline has been presumed to be increased left ventricular work due to arterial vasoconstriction, which lead to relative cardiac insufficiency and shift of the blood volume from the systemic to the pulmonary circulation (46, 69, 168 202, and others) In the rab*bit, the* pulmonary venous pressure is raised proportionally to the lung weight (68)

Kramer (1933) considered that the increased blood-volume in the lungs was attributable to a vasomotor redistribution of blood rather than to left ventricular failure.

The central nervous system is thought to play a part in the genesis of adrenaline lung oedema. Accordingly cervical vagotomy (167) or sympathectomy unilaterally or brain-stem lesions (99) protect against the development of pulmonary oedema. Diverse pharmacological preparations, such as chloral hydrate, barbiturates, heparin, morphine, palfium, phenergan, and others, have been found to exert some protective action against lung oedema (24 114, 167 200, 210) Stone & Loew (1949) however observed that the susceptibility to lung oedema after adrenaline increased when barbiturates had been given immediately before the injection. Analeptics *(167) and vagotomy (25)* increased the susceptibility to pulmonary oedema.

Bronchial constriction following large doses of adrenaline has been considered as a causative factor in pulmonary oedema *(25) Lung oedema develops less frequent*ly in tracheotomized than in non-tracheotomized experimental animals (243) The development of lung oedema after injection of adrenaline is counteracted by artificial respiration (80) and can be prevented by lowering the negative pressure in the chest (137)

cases without pulmonary oedema. The frequency of bleedings increased in *significantly with increasing uraemia*

Atelectasis was a common finding in blue-pink small lungs or lung lobes. Extensive changes were noted in 11 and less extensive changes in 10 cases. *The amount of pleural effusion in* these 21 rabbits averaged 25 (0—54) ml Atelectasis was commonest in rabbits with slight uraemia Local atelectasis occurred in rabbits with bronchitis *In the rabbit which had 637 ml of* ascitic fluid there were no atelectasis or other microscopical abnormalities.

An increase in the number of polymorphonuclear leucocytes was noticed in 10 cases. *Areas of purulent bronchitis* were seen in 5 and small pneumonic lesions in 2 cases. These animals had not been given antibiotics.

One rabbit which died shortly after the *infusion* had periarterial oedema

Bronchiolitis (Figs 18 and 19) was histologically *the most striking change* in these uraemic rabbits after Narkotal anaesthesia and was not noted in any other category of the animals in this investigation The lesions were *confined to the small bronchioles*

The bronchiolitis was characterized by destruction of the *epithelial cells* and splitting of the nuclei into minute fragments In some cases it involved only a few bronchioles or perhaps only a small part of the wall in a bronchiole *this finding is designated as "+* in the table. Fibrinous membranes were in a few cases seen *in the bronchioles*, extending towards the alveoli Hyaline membranes were faintly discernible in 1 case

Lungs from 108 uraemic non-overhydrated or overhydrated rabbits which had received 1 7 ml or 1.2 ml of Narkotal per kg body weight intravenously were examined microscopically *Bronchiolitis was present in 51 %* 45 animals had distinct and 11 had less marked changes Among the 45 rabbits with distinct bronchiolitis 44 % had pulmonary oedema Out of 63 rabbits *without or with insignificant* bronchiolitis 11 (17 %) had pulmonary oedema *7 out of these 11 died* spontaneously however within a few hours of the narcosis Bronchiolitis was not present in rabbits which died within 8 hours of the narcosis 21 rabbits *with pulmonary oedema survived* at least 20 hours after the narcosis 86 % had distinct bronchiolitis. Out of 77 rabbits without at least three criteria of pulmonary oedema 33 % had distinct bronchiolitis. Bronchiolitis was present in 33 % of 22 examined *animals which had had anuria for 2* hours before the narcosis, in 40 % of 20 animals with anuria for 24 hours, in 61 % of 33 animals with anuria for 48 hours, and in 58 % of 33 animals *with anuria for 72 hours.* There was no correlation between the duration *of anaesthesia and the occurrence of* bronchiolitis (r = 0 03 ± 0 10) nor between maximum depth of narcosis and the occurrence of bronchiolitis (r = 0 20 ± 0 09)

Whether the bronchiolitis was a parallel phenomenon or a prerequisite for *pulmonary oedema* is, thus, not clear

Survey See page 140

rabbits a) Among 10 animals which had been anuric for 3—4 hours before the injection of adrenaline, none died within 20 minutes and 2 within 65 minutes 8 were bled. b) Among 10 animals which had been anuric for 48 hours before injection of adrenaline 4 died within 20 minutes and none within 5 hours 6 were bled. c) Out of 10 animals which had been anuric for 72 hours before adrenaline injection 3 died within 20 minutes and 5 within 3 hours 2 were bled.

In the said groups the mean survival time was shortest (54 minutes) for the non-overhydrated animals with anuria for 72 hours and longest (206 minutes) for overhydrated animals with anuria for 3—4 hours (Tables 22 and 23)

Symptoms in vivo

For each group the results could have been grouped and reported under different headings, for instance pulmonary oedema—no pulmonary oedema slight uraemia—severe uraemia, over hydration,—non-overhydration. Since such an account would take up too much space, the groups will be brought together when they are similar or of little interest individually The frequency of pulmonary oedema in the different groups will be reported after the symptoms in vivo and autopsy findings.

Appearance and behaviour· Within a few minutes of the injection of adrenaline the animals became restless and moved about in the room all the time, often sniffing keenly and seemingly frightened. They had periods of tachypnoea and dyspnoea

The subsequent course varied individually Out of 70 rabbits, 13 died within 25 minutes and 4 within 1 hour of the injection, with frothy fluid gushing forth from the nose and the mouth the flow and the froth were less profuse in the overhydrated than in the non-overhydrated animals. In severely uraemic rabbits the course from injection up to death was quieter and the amount of frothy fluid less than in the slightly uraemic rabbits and the controls.

Convulsions immediately before death occurred in about half the cases and both in seemingly moribund and in seemingly alert animals. The occurrence of convulsions was not correlated to the occurrence of pulmonary oedema.

Weakness developed in about one-fourth of the rabbits within a few minutes of the injection of adrenaline and later in most of those which survived the first hour of restlessness death occurred inconspicuously in most of these animals. The weakness was particularly marked in highly uraemic rabbits In cases of great weakness it was very difficult to judge from the animal's condition whether or not it had pulmonary oedema. Even rabbits which survived until they were bled would sit still or lie flat on the floor during the last few hours before the bloodletting. Serum potassium was determined in 9 animals at the blood letting the levels did not differ significantly from those in anuric animals which had not had injections of adrenaline.

Respiratory rate In 7 normal rab-

Adrenaline did not induce pulmonary oedema in experiments with isolated lungs of rabbits (40) Other pharmacological preparations, for instance dinitrophenol, mercury compounds, and ammonia induced lung oedema in such experiments, which was interpreted as a result of permeability damage

Blood accumulating in the lungs has been noted in the initial stage of adrenaline-induced pulmonary oedema (211)

Wiggers produced lung oedema in dogs by infusion of adrenaline and saline (Chapter 6)

Own investigations

This series was used for the purpose of studying the effect of injection of adrenaline in control animals and non overhydrated and overhydrated rabbits with ligated ureters and uraemia of varying duration.

The series comprised 10 controls, and 60 rabbits whose ureters had been tied bilaterally The latter were divided into 3 groups of 20 animals adrenaline was injected 1—4 48, and 72 hours respectively after the operation In half the rabbits of each group Ringer's solution was infused intravenously in amounts equalling 15 % of the body weights for 1 1/2 hours. Adrenaline was injected 1 hour after the termination of infusion In the overhydrated rabbits the dose of adrenaline was proportioned to body weight after the infusion It was thus estimated that an approximately equal adrenaline concentration would be obtained in the body fluids of overhydrated and of non-overhydrated rabbits.

Accordingly overhydrated animals received a dose which was, on an average 15 % larger than that given to the non overhydrated ones.

Adrenaline was injected intramuscularly by Drenckhahn's (42) method. The dose was reduced, however to 1 mg per kg (1 ml 1 % adrenaline solution per kg) as preliminary experiments showed that in uraemic rabbits this dose produced pulmonary oedema in a desired frequency without too high mortality Before the injection checking was done by aspiration to make sure that the point of the needle was not in a blood vessel.

The rabbits were allowed to more freely in the room under continuous observation. Electrocardiograms were taken and the respiratory rate measured frequently after the injection. In the rabbits that died spontaneously chest radiographs were taken immediately after death Rabbits that lived for 5 hours after the injection were bled as soon as chest radiographs and electrocardiograms had been taken and the respiratory rate had been measured. The time of 5 hours was chosen because according to preliminary experiments, the effect of adrenaline usually begins to subside after that time.

Survival time

Out of 10 controls, 4 died spontaneously within 20 minutes and 4 within 5 hours 2 were bled after 5 hours.

The series of non-overhydrated anuric rabbits a) Out of 10 animals which had been anuric for 1—4 hours before the injection of adrenaline 3 died within 20 minutes and 1 within 70 minutes 6 were bled after 5 hours. b) Among 10 animals which had been anuric for 48 hours before the injection of adrenaline 3 died within 20 minutes and 4 within 3 hours 3 were bled. c) Among 10 animals which had been anuric for 72 hours before the injection of adrenaline 2 died within 20 minutes and 8 within 135 minutes.

The series of overhydrated anuric

rabbits a) Among 10 animals which had been anuric for 3—4 hours before the injection of adrenaline none died within 20 minutes and 2 within 65 minutes 8 were bled. b) Among 10 animals which had been anuric for 48 hours before injection of adrenaline, 4 died within 20 minutes and none within 5 hours 6 were bled c) Out of 10 animals which had been anuric for 72 hours before adrenaline injection, 3 died within 20 minutes and 5 within 3 hours 2 were bled.

In the said groups the mean survival time was shortest (54 minutes) for the non-overhydrated animals with anuria for 72 hours and longest (266 minutes) for overhydrated animals with anuria for 3—4 hours (Tables 22 and 23)

Symptoms in vivo

For each group the results could have been grouped and reported under different headings for instance pulmonary oedema—no pulmonary oedema, slight uraemia—severe uraemia, over hydration,—non-overhydration Since such an account would take up too much space, the groups will be brought together when they are similar or of little interest individually The frequency of pulmonary oedema in the different groups will be reported after the symptoms in vivo and autopsy findings.

Appearance and behaviour· Within a few minutes of the injection of adrenaline the animals became restless and moved about in the room all the time often sniffing keenly and seemingly frightened. They had periods of tachypnoea and dyspnoea.

The subsequent course varied individually Out of 70 rabbits, 13 died within 25 minutes and 4 within 1 hour of the injection, with frothy fluid gushing forth from the nose and the mouth the flow and the froth were less profuse in the overhydrated than in the non-overhydrated animals. In severely uraemic rabbits the course from injection up to death was quieter and the amount of frothy fluid less than in the slightly uraemic rabbits and the controls.

Convulsions immediately before death occurred in about half the cases and both in seemingly moribund and in seemingly alert animals. The occurrence of convulsions was not correlated to the occurrence of pulmonary oedema.

Weakness developed in about one-fourth of the rabbits within a few minutes of the injection of adrenaline and later in most of those which survived the first hour of restlessness death occurred inconspicuously in most of these animals. The weakness was particularly marked in highly uraemic rabbits. In cases of great weakness it was very difficult to judge from the animal's condition whether or not it had pulmonary oedema. Even rabbits which survived until they were bled would sit still or lie flat on the floor during the last few hours before the bloodletting. *Serum potassium was* determined in 9 animals at the blood letting· the levels did not differ significantly from those in anuric animals which had not had injections of adrenaline.

Respiratory rate In 7 normal rab-

Adrenaline did not induce pulmonary *oedema in experiments with isolated lungs* of rabbits (40) Other pharmacological preparations, for instance dinitrophenol, mercury compounds, and ammonia induced lung oedema in such experiments which was interpreted as a result of permeability damage.

Blood accumulating in the lungs has been noted in the initial stage of adrenaline-induced pulmonary oedema (211)

Wiggers produced lung oedema in dogs by infusion of adrenaline and saline (Chapter 6)

Own investigations

This series was used for the purpose of studying the effect of injection of adrenaline in control animals and non overhydrated and overhydrated rabbits with *ligated ureters and uraemia of varying duration*

The series comprised 10 controls, and 60 rabbits whose ureters had been tied bilaterally The latter were divided into 3 groups of 20 animals adrenaline was injected 1—4 48, and 72 hours, respectively after the operation In half the rabbits of each group Ringer s solution was infused intravenously in amounts equalling 15 % of the body weights for 1 1/2 hours Adrenaline was injected 1 hour after the termination of infusion. In the overhydrated rabbits the dose of adrenaline was proportioned to body weight after the infusion It was thus estimated that an approximately equal adrenaline concentration would be obtained in the body fluids of overhydrated and of non-overhydrated rabbits.

Accordingly overhydrated animals received a dose which was on an average 15 % larger *than that given to the* non-overhydrated ones.

Adrenaline was injected intramuscularly *by* Drenckhahn s (42) method. The dose was reduced, however to 1 mg per kg (1 ml 1 % adrenaline solution per kg) as preliminary experiments showed that in uraemic rabbits this dose produced pulmonary oedema in a desired frequency without too high mortality Before the injection checking was done by aspiration to make sure that the point of the needle was not in a blood vessel.

The rabbits were allowed to more freely in the room under continuous observation Electrocardiograms were taken and the respiratory rate measured frequently after the injection In the rabbits that died spontaneously chest radiographs were taken immediately after death. Rabbits *that lived for 5 hours after the injection* were bled as soon as chest radiographs and electrocardiograms had been taken and *the respiratory rate had been measured.* The time of 5 hours was chosen because *according to preliminary experiments, the effect of adrenaline usually begins to subside* after that time

Survival time

Out of 10 controls, 4 died spontaneously *within 20 minutes and 4 within* 5 hours 2 were bled after 5 hours.

The series of non-overhydrated anuric rabbits a) Out of 10 animals which had been anuric for 1—4 hours *before the injection of adrenaline* 3 died within 20 minutes and 1 within 70 minutes 6 were bled after 5 hours. b) Among 10 animals which had been anuric for 48 hours before the injec*tion of adrenaline 3 died* within 20 minutes and 4 within 5 hours 3 were bled c) Among 10 animals which had been anuric for 72 hours before the injection of adrenaline 2 died within 20 minutes and 8 within 135 minutes.

The series of overhydrated anuric

rabbits a) Among 10 animals which had been anuric for 3—4 hours before the injection of adrenaline, none died within 20 minutes and 2 within 65 minutes 8 were bled. b) Among 10 animals which had been anuric for 48 hours before injection of adrenaline 4 died within 20 minutes and none within 5 hours 6 were bled. c) Out of 10 animals which had been anuric for 72 hours before adrenaline injection, 3 died within 20 minutes and 5 within 3 hours 2 were bled.

In the said groups the mean survival time was shortest (54 minutes) for the non-overhydrated animals with anuria for 72 hours and longest (266 minutes) for overhydrated animals with anuria for 3—4 hours (Tables 22 and 23)

Symptoms in vivo

For each group the results could have been grouped and reported under different headings, for instance pulmonary oedema—no pulmonary oedema slight uraemia—severe uraemia, over hydration,—non-overhydration. Since such an account would take up too much space the groups will be brought together when they are similar or of little interest individually The frequency of pulmonary oedema in the different groups will be reported after the symptoms in vivo and autopsy findings.

Appearance and behaviour: Within a few minutes of the injection of adrenaline the animals became restless and moved about in the room all the time often sniffing keenly and seemingly frightened. They had periods of tachypnoea and dyspnoea

The subsequent course varied individually Out of 70 rabbits, 13 died within 25 minutes and 4 within 1 hour of the injection, with frothy fluid gushing forth from the nose and the mouth the flow and the froth were less profuse in the overhydrated than in the non-overhydrated animals. In severely uraemic rabbits the course from injection up to death was quieter and the amount of frothy fluid less than in the slightly uraemic rabbits and the controls.

Convulsions immediately before death occurred in about half the cases and both in seemingly moribund and in seemingly alert animals. The occurrence of convulsions was not correlated to the occurrence of pulmonary oedema.

Weakness developed in about one-fourth of the rabbits within a few minutes of the injection of adrenaline and later in most of those which survived the first hour of restlessness death occurred inconspicuously in most of these animals. The weakness was particularly marked in highly uraemic rabbits. In cases of great weakness it was very difficult to judge from the animal s condition whether or not it had pulmonary oedema Even rabbits which survived until they were bled would sit still or lie flat on the floor during the last few hours before the bloodletting Serum-potassium was determined in 9 animals at the bloodletting the levels did not differ significantly *from* those in anuric animals which had not had injections of adrenaline.

Respiratory rate In 7 normal rab-

bits the respiratory rate was measured at short intervals from the adrenaline injection up to death which occurred within 35—310 minutes after the injection Four of these animals had at least three criteria of pulmonary oedema at autopsy The respiratory rate varied greatly from minute to minute but seldom exceeded 200 per minute. Attacks of dyspnoea occurred. Despite these respiratory studies, however it was not possible to establish with any degree of certainty which animals had or were to develop pulmonary oedema

In 28 anuric rabbits, which later on did not exhibit pulmonary oedema the respiratory rate about 30 minutes after adrenaline injection averaged 109 (36—320) per minute. In 27 rabbits which later on exhibited pulmonary oedema the respiratory rate averaged 187 (72—380) per minute The severely uraemic animals with pulmonary oedema had a respiratory rate as high as 242 (120—380) per minute.

In 23 severely ill uraemic rabbits the respiratory rate was recorded within 10 minutes before spontaneous death In 15 rabbits with pulmonary oedema it averaged 228 (92—380) and in 8 rabbits without pulmonary oedema 97 (26—200) per minute.

The respiratory rate was thus of some aid in establishing or excluding the diagnosis of pulmonary oedema following adrenaline injection in all the groups and hence in severely uraemic animals as well. However the individual variations in the frequency of tachypnoea and dyspnoea reduced the diagnostic value of the symptoms in the individual case.

E.C.G and heart rate In 10 normal and 4 anuric rabbits electrocardiograms were taken at short intervals up to death Bradycardia arose within some minutes of the adrenaline injection. After a further few minutes deformation of the QRS-complex and/or arrhythmia appeared. Out of 8 rabbits that died in this stage, 4 had pulmonary oedema In the 6 animals that lived longer arrhythmia and QRS deformation also appeared but the symptoms subsided or disappeared later on and the bradycardia changed into tachycardia Tachycardia and flat or negative T waves persisted in many cases up to a few minutes before spontaneous death when the frequency of severe electrocardiographic changes increased again

Thus in this group despite frequent recordings, the development of pulmonary oedema could not be predicted with the aid of E.C.G The animals could have severe E.C.G disturbances for several hours before spontaneous death and yet exhibit no evidence of pulmonary oedema at autopsy All those that died spontaneously had severe E.C.G abnormalities immediately before death.

Before the adrenaline injection 2? out of 27 subsequently bled rabbits had normal electrocardiograms, whereas in 5 the T waves were absent Five hours after the adrenaline injection the E.C.G showed no abnormalities in 6 absent T waves in 5 negative T waves in 17 and deformation of the QRS-complex in 1

The heart rate of the 27 bled rabbits averaged before the adrenaline injec

tion 237 (120—330) and 5 hours after the injection 235 (136—275) beats per minute. 7 rabbits with pulmonary oedema had immediately before the bloodletting an average heart rate of 235 (170—273) beats per minute.

All the rabbits with pulmonary oedema had more or less severe E.C.G changes, but, again, many animals without pulmonary oedema had similar changes. The heart rate showed the same variations in animals with and without pulmonary oedema. Rabbits in all stages of uraemia had severe E.C.G disturbances after the adrenaline injection in the highly uraemic ones the changes disappeared less readily or persisted. The E.C.G showed the same disturbances in the overhydrated as in the non-overhydrated animals.

Thus, the E.C.G. and the heart rate were of little diagnostic value in the adrenaline series.

Heart size. The series consisted of 70 animals. Before the adrenaline injection the heart-size could be estimated radiographically in 62 in 8 the heart could not be assessed for technical reasons or because pictures were missing. In 55 of the 62 animals the heart size was normal, whereas 8 (all overhydrated) had slight cardiac enlargement.

After the adrenaline injection immediately before bloodletting spontaneous death, and autopsy respectively (Tables 22 and 23) the results for the 70 rabbits were as follows The heart was markedly enlarged in 3, moderately enlarged in 13 slightly enlarged in 19 and of normal size in 24 cases the heart size could not be assessed in 10 rabbits in which pulmonary oedema made definition of the heart impossible the radiograph was missing in 1 case. If the 70 animals (10 normal and 60 uraemic) are grouped by overhydration or non-overhydration and severe, slight, or no uraemia, the following figures will be obtained In 26 of 30 overhydrated uraemic rabbits the heart could be defined 72 % had cardiac enlargement. In 34 out of 40 non-overhydrated rabbits (30 uraemic and 10 normal) the heart could be defined 50 % had cardiac enlargement. In 24 of 30 normal or slightly uraemic (anuria for less than 24 hours) rabbits the heart could be defined 71 % had cardiac enlargement. In 35 out of 40 highly uraemic (anuria for 48 hours) rabbits the heart could be defined 51 % had cardiac enlargement. Cardiac enlargement was commonest in overhydrated rabbits with slight uraemia.

14 out of 70 rabbits were not examined until immediately after death in 8 the heart was of normal size, in 3 slightly enlarged, and in 3 moderately enlarged. If these are taken into account, 63 % of 24 animals with pulmonary oedema had no or slight cardiac enlargement and 56 % of 16 animals with marked to moderate cardiac enlargement had pulmonary oedema.

The ratio pulmonary oedema/cardiac enlargement could be studied in 25 bled animals (Tables 22 and 23) 9 had normal heart size (2 had pulmonary oedema) 5 had slightly enlarged heart (2 had pulmonary oedema) 9 had moderately enlarged heart (2 had pul

Table 22 *Results after intramuscular injection of adrenaline 1 mg per kg body-weight in normal and in anuric rabbits whose ureters had been tied 1—4 48 and 72 hours respectively before the injection. I = at least three criteria of pulmonary oedema S = spontaneous death*

		Lung weight g/kg body weight	Water content in lung	Pulmonary oedema by gross observation	Frothy fluid in the air ways	Alveolar oedema	X ray Lung changes	X ray Cardiac enlargement	Pleural fluid ml	Ascites ml	N P N mg per 100 ml	Length of survival after adrenaline injection	
I Adrenaline in normal rabbits	L	10.51	88.7	+++	+++	+	+++	+++	0	12	85	160	S
	I	12.0	88.0	+++	+++	+	0	0	0	2	68	8	S
	L	10.28	87.0	++	+++	++	++	?	0	0	44	13	S
	I	9.78	80.2	++	+++	+++	++	++	0	0	—	35	S
	I	8.10	83.6	+++	+	++	++	++	4	0	70	310	S
	I	8.76	78.7	+	0	+	0	0	0	0	58	325	
		5.11	81.5	+	0	+	0	+	0	11	78	185	S
		5.01	80.0	0	0	—	0	+	0	0	50	3	S
		4.11	81.7	0	0	—	0	+	3	2	42	320	
		4.11	81.7	+	0	—	0	+	1	0	34	3	S
Mean		8.20	83.8										
Mean									1	3	60		
II Adrenaline 1—4 hours after ligation of ureters	L	14.51	89.7	+++	++	+	+++	?	5	0	96	330	
	L	12.61	86.8	+++	+++	++	++	++	0	0	60	15	S
	L	12.20	88.3	+++	++	+++	++	+	3	1	62	300	
	L	11.08	86.0	+++	+++	+++	++	?	1	0	76	15	S
	L	8.53	86.6	++	+	+	+	+++	4	0	81	330	
		78	85.2	0	0	0	0	0	0	0	67	70	S
		5.17	87.9	0	0	0	—	—	0	0	60	10	S
		3.78	81.7	0	0	—	0	0	0	0	65	315	
		3.77	81.0	0	0	—	0	0	0	0	60	305	
		3.76	78.5	0	0	—	0	0	0	0	97	330	
Mean		8.08	81.8										
Mean									1	0	72		

III. Adrenaline injection 48 hours after ligation of reters	L	16.39	83.0	+++	+++	+++	++	+	11	160	258	15	5
	L	14.21	86.6	+++	+++	+++	+++	?	0	3	186	15	5
	L	11.06	88.4	+++	+	++	?	?	5	3	305	175	5
	L	6.60	83.5	+	●	+	+	0	0	[illegible]	198	30	5
		5.57	80.9	0	0	0	0	0	0	60	226	30	5
		5.43	81.8	●	0	0	0	0	[illegible]	8	183	7	5
		4.07	83.3	0	0	—	0	+	1	[illegible]	163	338	
		4.62	79.7	0	0	—	0	0	0	[illegible]	223	125	5
		3.73	81.2	0	0	—	0	0	0	3	196	310	
		3.38	80.2	0	0	—	0	0	0	77	216	300	
									3	23	303		
Mean		7.15	83.1										
II. Adrenaline injection 72 hours after ligation of ureters	L	11.45	86.1	+++	+	++	++	0	1	120	231	13	5
	L	11.27	68.2	+++	+	+++	+	+	0	68	367	21	5
	L	10.63	87.0	+++	+++	+	++	+	6	123	313	25	5
		6.16	80.3	0	0	0	0	+	3	165	238	165	5
		8.4	81.5	0	0	+	0	0	[illegible]	00	209	23	5
		5.37	81.2	0	0	0	0	0	1	35	373	125	5
		4.98	82.3	0	0	0	0	0	4	18	301	37	5
		4.80	80.8	●	0	0	●	++	1	2	284	135	5
		1.38	82.8	0	●	—	0	+	4	31	273	35	5
		3.95	79.6	0	●	—	●	0	[illegible]	74	265	20	5
									3	73	276		
Mean		6.79	83.9										

Table 22. *Results after intramuscular injection of adrenaline 1 mg per kg body weight in normal and in anuric rabbits whose ureters had been tied 1—4 48 and 72 hours respectively before the injection L=at least three criteria of pulmonary oedema S=spontaneous death*

		Lung weight g/kg body weight	Water content of lung %	Pulmonary oedema by gross observation	Frothy fluid in the air way	Alveolar oedema	X-ray: Lung changes	X-ray: Cardiac enlargement	Pleural fluid ml	Ascites ml	N P N mg per 100 ml	Length of survival after adrenaline injection	
I Adrenaline in normal rabbit	L	10.51	88.7	+++	+++	+	+++	+++	0	12	85	160	S
	L	12.70	88.0	+++	+++	+	0	0	0	2	08	8	S
	I	10.28	87.0	++	+++	++	++	?	0	0	44	13	S
	L	9 78	86.2	++	+++	+++	++	++	0	0	—	35	S
	I	8.10	83.6	+++	+	++	++	++	1	0	76	310	S
	I	0.26	78.7	+	0	+	0	0	0	0	58	325	
		5 11	81.5	+	0	+	0	+	0	11	78	185	S
		5 01	80 9	0	0	—	0	+	0	0	50	3	S
		4 11	81 7	0	0	—	0	+	3	2	42	370	
		1 11	81 7	+	0	—	0	+	1	0	31	3	S
Mean		8.20	83 8						1	3	50		
II Adrenaline 1—4 hours after ligation of ureters	L	14 51	80 7	+++	++	+	+++	?	5	0	96	330	
	I	12.01	86 8	+++	+++	++	++	++	0	0	90	15	S
	L	12.20	88.3	+++	++	+++	++	+	3	1	92	300	
	I	11 08	86 9	+++	+++	+++	++	?	1	0	70	15	S
	I	8.33	86.6	++	+	+	+	+++	1	0	81	330	
		5.38	8. 2	0	0	0	0	0	0	0	97	70	S
		. 17	82 0	0	0	0	—	—	0	0	60	10	S
		3.78	81 7	0	0	—	0	0	0	0	55	315	
		3 77	81.0	0	0	—	0	0	0	0	90	305	
		3. 0	8 5	0	0	—	0	0	0	0	97	330	
Mean		8 08	81.8						1	0	72		

III. Adrenaline injection 72 hours after ligation of ureters	L	16.33	88.9	+++	●	+	+++	?	15	36	201	100	5
	L	14.15	80.3	+++	++	+	+++	+	5	29	210	29	5
	L	11.93	86.9	+++	+++	+	++	0	0	145	270	5	5
	L	8.48	84.7	++	0	0	++	+	1	322	331	106	5
	L	6.67	81.8	++	0	+	+	0	5	10	260	300	
		5.38	84.0	+	0	0	●	●	1	13	268	300	
		4.97	81.6	0	0	—	++	0	13	116	248	160	5
		4.91	83.8	0	0	—	●	+	2	257	210	35	5
		1.83	82.3	0	0	—	●	●	1	358	301	90	5
		4.63	83.2	0	●	—	0	+	20	116	222	17	5
									0	140	790		
Mean		8.12	85.0										

Table 23. *Results after infusion of Ringer's solution 15 % of the body-weight for 1 1/2 hours and injection of adrenaline intramuscularly 1 mg per kg body-weight in rabbits whose ureters had been tied 3—4, 48 and 72 hours respectively before the injection. L = at least three criteria of pulmonary oedema. S = spontaneous death*

		Lung weight g/kg body weight	Water content of lung	Pulmonary oedema by gross inspection	Frothy fluid in the airways	Alveolar oedema	X-ray: Lungs	X-ray: Cardiac enlargement	Pleural fluid ml	Ascites ml	NPN mg per 100 ml	Length of survival after adrenaline injection minutes	
I Adrenalin injection 3—4 hours after ligation of ureters	L	10.97	87.7	+++	++	+	+++	?	16	12	75	325	
	L	10.70	90.3	+++	++	+	++	+++	28	38	80	310	
	L	8.80	87.5	+++	++	+	+	++	33	68	82	315	
	L	7.77	84.2	++	++	++	+++	++	2	12	80	60	S
	L	6.55	85.9	++	++	+	+++	?	13	48	75	65	S
		5.02	85.0	0	0	—	0	++	3	10	90	300	
	L	5.77	83.7	+	0	+	+	+	0	35	78	320	
		5.08	85.6	0	0	—	0	+	16	11	90	330	
		5.18	81.1	0	0	—	0	0	25	80	90	335	
		5.03	83.3	0	0	—	0	+	13	58	67	300	
Mean		7.28	85.5						16	38	71		
II Adrenalin injection 48 hours after ligation of ureters	L	10.72	85.0	+++	+++	0	+++	?	1	30	108	15	S
	L	13.48	88.1	+++	+++	0	+++	+	2	58	180	5	S
	L	13.09	89.1	+++	+++	0	+++	?	3	23	175	20	S
	L	12.07	87.8	+++	+++	0	+++	0	2	4	107	15	S
	L	5.73	81.5	0	0	—	+	++	7	35	108	315	
		5.48	85.5	0	0	—	0	++	4	115	?01	320	
		5.37	83.0	0	0	—	0	++	8	38	172	300	
		5.03	85.0	+	0	0	0	++	1	75	105	315	
		1.77	85.0	0	0	0	0	++	5	18	162	330	
		1.50	83.8	0	0	—	0	++	20	51	220	310	
Mean		9.02	85.0						6	45	152		

30 animals but the number was not sufficient in 1 non-overhydrated and 8 overhydrated animals. Four animals in which pulmonary oedema was established by at least three other criteria had normal water content. One rabbit had a water content of only 78.7 % but the lung weight, gross findings (numerous small haemorrhages in the lung parenchyma) and microscopical picture established the diagnosis of pulmonary oedema.

Microscopical alveolar oedema was present in 30 out of 46 animals, including 28 which had at least two other criteria of pulmonary oedema. Five overhydrated animals with pulmonary oedema by four or five other criteria had no alveolar oedema (similar observations have been described earlier by Visscher et al.)

In 24 rabbits microscopical examination was not performed, as autopsy showed pale-red air-filled lungs without frothy fluid and of normal weight. Their lung weights had been calculated per kg body weight on the day of injection of adrenaline. After the experiments, however, when calculated per kg body weight on the day of ureteral ligation, the lung weights were higher than normal in 2 of these cases.

The commonest microscopical findings in the rabbits with pulmonary oedema evidenced by at least three criteria were clearly visible alveolar oedema, marked hyperaemia (mentioned earlier by Poulsen [211]), moderate amounts of red cells in the alveoli, an increase in the numbers of alveolar phagocytes, and periarterial interstitial oedema.

There were many exceptions, possibly attributable to overhydration and the extent of the oedema. In the series of non-overhydrated animals with pulmonary oedema evidenced by at least three criteria, alveolar oedema graded as +++ was noted in 0 cases, ++ in 5, and + in 7 cases. In the series of overhydrated animals with pulmonary oedema by at least three criteria, alveolar oedema +++ was present in none, ++ in 1 case, + in 0 cases. In 5 there was no alveolar oedema and in 1 case the preparation was lost.

The investigation shows that the frequency of distinct alveolar oedema was much lower in overhydrated than in non-overhydrated rabbits. In cases of slight pulmonary oedema the alveolar oedema was often more distinct and more often seen occurring in patches than in cases of severe pulmonary oedema in which it could be indistinct and continuous, and in which the whole microscopical picture looked almost normal.

Periarterial interstitial oedema was not seen in every rabbit with pulmonary oedema. In those that had red dots on the lung surfaces focal haemorrhages were seen microscopically. Bluish-pink lungs were slightly atelectatic. Nor was there any thickening of the alveolar wall in lungs with a high water content. Characteristic of the whole series was that bronchial changes were absent, atelectatic areas uncommon, and haemorrhages rare. Leucocytes were seen diffusely in increased numbers in scarcely half the cases; foci of infection were not seen.

monary oedema) and 2 had markedly enlarged heart (2 had pulmonary oedema) Thus there was no high degree of correlation between heart size and pulmonary changes.

Radiographic changes With only one exception all the examined animals (63 out of 70) had radiographically normal lungs immediately before the adrenaline injection. This one rabbit which was overhydrated developed highly frothy pulmonary oedema immediately after the injection After the adrenaline injection 32 animals (46 %) developed pulmonary changes and in 36 (51 %) X ray of the lungs was normal the picture could not be assessed in the remaining 2 cases (3 %)

The result of the radiographic examination showed good agreement with other criteria of lung oedema (Tables 22 and 23) A noteworthy exception was a rabbit in which the lungs were normal on X ray at the moment of death—8 minutes after the adrenaline injection — although frothy fluid was gushing forth from its nose and mouth at autopsy 5 minutes later the animal was found to have pulmonary oedema by all postmortem criteria In another animal the lungs in vivo showed moderate radiographic changes which could be interpreted as oedema autopsy a few minutes later revealed extensive bilateral atelectasis and only sparse pleural and ascitic fluid.

Postmortem findings

Gross observation at autopsy showed evidence of pulmonary oedema in 37 out of 70 rabbits. In 30 cases there was distinct evidence in the form of redish pink blotches or spots over the lung surfaces and profuse flow of fluid on section of the lung In the other 7 rabbits there was slight evidence of pulmonary oedema namely numerous red dots changing into small red patches in the hilar region Both large and small lesions showed a clear tendency to accumulate at the hilus and the base. In 9 rabbits a few red dots were seen on the lung surfaces and 5 had small bluish pink lungs.

In 34 of the above mentioned 37 rabbits the macroscopical diagnosis of pulmonary oedema was supported by at least two other criteria One rabbit had no gross evidence of lung oedema of the type discussed here although three other criteria supported the diagnosis

Frothy fluid in the air ways was found in 27 animals, all of which had pulmonary oedema evidenced by at least four criteria In 7 animals with pulmonary oedema by at least three criteria there was no frothy fluid in the air ways.

The lung weight in 36 of 70 animals exceeded the upper limit of normal range. The diagnosis of pulmonary oedema was supported by at least two other criteria in 34 of these 36 (Tables 22 and 23)

The water content of the lungs exceeded *the upper limit of normal range* in 56 % (39 of 70 animals) the figure for the overhydrated rabbits was 7· % and for the non-overhydrated one 40% (Tables 22 and 23) *An adequate* number of criteria *for the diagnosis* of pulmonary *oedema were fulfilled in*

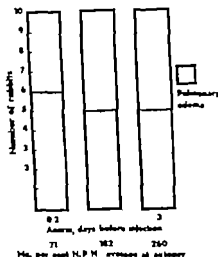


Diagram 11 Frequency of pulmonary edema after intravenous infusion of Ringer' solution, 15 % of the body-weight for 1 1/2 hours, and intramuscular injection of adrenaline, 1 mg per kg body-weight, in rabbits which had been anuric for 0.2, 2, and 3 days, respectively before the injection.

of adrenaline in 6 of 10 controls, in 5 of 10 rabbits with anuria for 1—4 hours, in 4 of 10 rabbits with anuria for 48 hours, and in 3 of 10 rabbits with anuria for 72 hours.

Overhydrated rabbits (Table 23 and Diagram 11) Pulmonary oedema as defined here occurred after injection of adrenaline in 6 of 10 animals with anuria for 1—4 hours, in 5 of 10 with anuria *for 48 hours, and in 5 of 10* with anuria for 72 hours.

Thus, the frequency of adrenaline-induced pulmonary oedema in the rabbits whose ureters had been ligated decreased slightly with increasing uraemia, but the differences between slightly uraemic and highly uraemic animals is not statistically significant in either the overhydrated or the non-overhydrated series.

Pulmonary oedema and overhydration

The frequency of pulmonary oedema was higher in overhydrated than in non-overhydrated animals, but the difference is not significant and it was difficult to render the two groups comparable. The adrenaline concentration in blood and tissues can have differed between the two groups because of a larger fluid space (> 15 %) and a higher dose (15 %) in the overhydrated animals, different rate of absorption for the adrenaline injected intramuscularly differences in circulatory rate, and other factors.

Survival time in relation to frequency of pulmonary oedema and degree of uraemia

Among the 70 rabbits in the series, 7 died within 10 minutes of the injection of adrenaline 3 of these 7 had pulmonary oedema according to the definition used here (43 %) 22 died spontaneously within 11—60 minutes of the injection 15 of these had pulmonary oedema (68 %) 14 died spontaneously within 61—310 minutes of the injection 6 of these had pulmonary oedema (43 %) 27 rabbits were bled within 5 hours of the injection 10 of these had pulmonary oedema (37 %) Cases of slight transient pulmonary oedema can of course have been missed and left out of the records.

Among those that died spontaneously without pulmonary oedema, 11 had been anuric for 72 hours, 3 for 48

Criteria of pulmonary oedema

The diagnosis of pulmonary oedema following injection of adrenaline in uraemic rabbits (Diagram 18 Tables 23 and 24) could in most cases be based on the correlation obtained between the methods of examination The diagnosis was in 19 cases based on six positive criteria in 10 cases on five, in 3 cases on four and in 2 cases on three criteria One X ray examination and one microscopical examination failed

In 36 cases the number of positive criteria was less than three, and it was therefore considered that pulmonary oedema was not present In 23 of these cases there were no positive criteria in 8 there was one criterion and in 5 there were two criteria One X ray examination failed.

In 23 cases the lungs were not examined microscopically In 16 of them there were no criteria in 5 there was one, and in 2 there were two criteria of slight pulmonary oedema (in the series of overhydrated animals with anuria for 3—4 hours) If alveolar oedema had been found in these 2 cases the number of criteria would thus have been sufficient to establish the diagnosis of pulmonary oedema This, however would have had very little influence on the conclusions concerning the relationship between the frequency of pulmonary oedema and the degree of uraemia on one hand and overhydration on the other

Atelectasis bleedings, and pneumonia were seldom seen. In 4 overhydrated rabbits alveolar oedema was absent although they had severe pulmonary

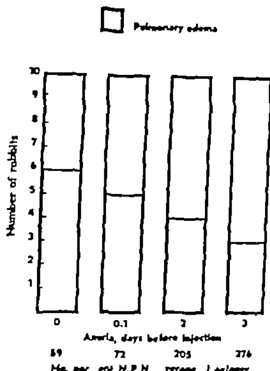


Diagram 10. Occurrence of pulmonary edema after intramuscular injection of epinephrine 1 mg per kg body weight, in normal rabbits and rabbits, which had been anuric for 0 1 2, and 3 days, respectively before the injection.

oedema evidenced by all the other criteria similar observations have been made earlier by for instance Visscher et al. Four overhydrated rabbits with pulmonary oedema by at least four criteria had no frothy fluid in the air ways profuse amounts of secretion were seen however The differential diagnosis was difficult in these cases.

Pulmonary oedema and degree of uraemia

The control series and the non overhydrated anuric rabbits (Table 22 and Diagram 10) Pulmonary oedema as defined here occurred after injection

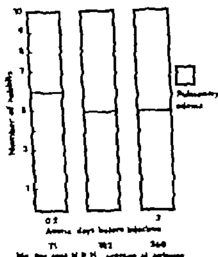


Diagram 11 Frequency of pulmonary edema after intravenous infusion of Ringer' solution, 15 % of the body-weight for 1 ½ hours, and intramuscular injection of adrenaline, 1 mg per kg body-weight, in rabbits which had been anuric for 0.2, 2, and 3 days, respectively befor the injection.

of adrenaline in 6 of 10 controls, in 5 of 10 rabbits with anuria for 1—4 hours, in 4 of 10 rabbits with anuria for 48 hours, and in 3 of 10 rabbits with anuria for 72 hours.

Overhydrated rabbits (Table 23 and *Diagram 11*) Pulmonary oedema as defined here occurred after injection of adrenaline in 6 of 10 animals with anuria for 1—4 hours, in 5 of 10 with anuria for 48 hours, and in 5 of 10 with anuria for 72 hours.

Thus, *the frequency of adrenaline induced pulmonary oedema in the rabbits whose ureters had been ligated decreased slightly with increasing uraemia, but the differences between slightly uraemic and highly uraemic animals is not statistically significant* in either the overhydrated or the non-overhydrated series.

Pulmonary oedema and overhydration

The frequency of pulmonary oedema was higher in overhydrated than in non-overhydrated animals, but the difference is not significant and it was difficult to render the two groups comparable. The adrenaline concentration in blood and tissues can have differed between the two groups because of a larger fluid space (> 15 %) and a higher dose (15 %) in the overhydrated animals, different rate of absorption for the adrenaline injected intramuscularly differences in circulatory rate, and other factors.

Survival time in relation to frequency of pulmonary oedema and degree of uraemia

Among the 70 rabbits in the series, 7 died within 10 minutes of the injection of adrenaline 3 of these 7 had pulmonary oedema according to the definition used here (43 %) 22 died spontaneously within 11—60 minutes of the injection 15 of these had pulmonary oedema (68 %) 14 died spontaneously within 61—310 *minutes of* the injection 6 of these had pulmonary oedema (43 %) 27 rabbits were bled within 5 hours of the injection 10 of these had pulmonary oedema (37 %) *Cases of slight transient pulmonary oedema can of course have* been missed and left out of the records.

Among those that died spontaneously without pulmonary oedema 11 had been anuric for 72 hours, 3 for 48

Criteria of pulmonary oedema

The diagnosis of pulmonary oedema following injection of adrenaline in uraemic rabbits (Diagram 18 Tables 23 and 24) could in most cases be bas ed on the correlation obtained between the methods of examination The diag nosis was in 19 cases based on six positive *criteria, in 10 cases on five* in 3 cases on four and in 2 cases on *three criteria* One X ray examination and one microscopical examination failed.

In 36 cases the number of positive criteria was less than three, and it was therefore considered that pulmonary oedema was not present. In 23 of these cases there were no positive criteria in 8 there was one criterion and in 5 there were two criteria One X ray exa mination failed.

In 23 cases the lungs were not exa mined microscopically In 16 of them *there* were no criteria in 5 there was one, and in 2 there were two criteria of slight pulmonary oedema (in the series of overhydrated animals with anuria for 3—4 hours) If alveolar oedema had been found in these 2 cases the number of criteria would thus have been sufficient to establish the diagnosis of pulmonary oedema This, however would have had very little influence on the conclusions con cerning the relationship between the frequency of pulmonary oedema and the degree of uraemia on one hand and overhydration on the other

Atelectasis, bleedings, and pneumo- nia were seldom seen In 4 overhydrat ed rabbits *alveolar oedema was absent* although they had severe pulmonary

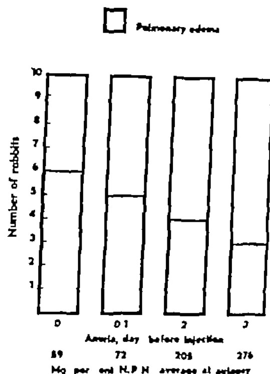


Diagram 10 Occurrence f pulmonary edema after intramuscular injection of epinephrine 1 mg per kg body weight, in normal rabbits and rabbits, which had been anuric for 0 1 2, and 3 days, respectively before the in jection.

oedema evidenced by all the other cri teria similar observations have been made earlier by for instance Visscher et al Four overhydrated rabbits with pulmonary oedema by at least four criteria had no frothy fluid in the air ways profuse amounts of secre tion were seen however The differen tial diagnosis was difficult in these cases.

Pulmonary oedema and degree of uraemia

The control series and the non-over hydrated anuric rabbits (Table 22 and *Diagram 10)* Pulmonary oedema as defined here occurred after injection

CHAPTER [illegible]

Bilateral vagotomy

Many surveys of the extensive literature on vagotomy have been presented (e.g. 90, 158, 168 214 225 246) Most of the experiments were carried out in rabbits, rats, and guinea pigs. In dogs and cats, on the contrary vagotomy permits indefinite survival (214) The following short survey of the literature will serve only as a background to my own experiments reported in this section. The account refers to vagotomy without previous overhydration and vagotomy in overhydrated animals.

a) *Vagotomy* After bilateral vagotomy in experimental animals, Lower (1664) noted death from dyspnoea, Vieussens (1715) accumulations of blood in the lungs, and Valsalva (1740) blood stained froth in the mouth Haller (1746—1760) ascribed the pulmonary changes to aspiration of gastric contents. Legallois (1812) suggested that the cause of death would be asphyxia due to reduced glottis, hyperaemia in the lungs, and/or the presence of serous fluid in the bronchi. Traube (1846) showed that tracheotomy protected rabbits against vagotomy oedema, concluding that vocal-cord paralysis was the mechanism of the lung lesion. Schiff (1850) in contradiction of Traube, found that pulmonary oedema occurred even after tracheotomy and did not note any lung lesions after bilateral division of the recurrent laryngeal nerve. Schiff formed the hypothesis of pulmonary vasomotor paralysis whereas Claude Bernard (1858) ascribed the lesions to altered respiratory dynamics Löwit (1893) did not find any rise in left atrial pressure and concluded that the lung oedema was brought about by vasodilatation with increased accumulation of blood and increased vascular permeability Shafer (1920) noted pulmonary oedema in association with slow asphyxia without vagotomy and suggested that this was the causative factor in the lung oedema following vagotomy Farber (1937) found that tracheotomy and artificial respiration did not protect rabbits against vagotomy oedema. On direct inspection he noted that the lung lesion began as petechia which gradually fused into large areas showing red discoloration which at autopsy were found to be consolidated areas. He considered that vagal denervation increased the permeability of the lung capillaries. Lorber (1939) found that with careful bronchial toilet after vagotomy outspoken lung oedema did not occur He suggested that circulatory failure in association with anoxia could be a factor involved in vagotomy oedema.

Short (1944) showed that tracheotomy alone without vagotomy can give rise to stagnation of secretion in the bronchi and cause slight pulmonary oedema. Reichsman (1946) found that the inspiratory phase was prolonged after vagotomy and that even with other techniques impeded inspiration can cause pulmonary oedema. This was confirmed in experiments by

hours, and 2 for a few hours, while 3 belonged to the control group

Thus, among highly uraemic animals overhydrated as well as non overhydrated ones the number of those that died spontaneously without pulmonary oedema was greater than it was among slightly uraemic animals The explanation could be that in highly uraemic animals death is more often due to some adrenaline effect other than pulmonary oedema In interpreting the results of the adrenaline series it is therefore difficult to determine whether highly uraemic animals have *an increased tendency toward pulmonary* oedema

Survey See page 144

CHAPTER 9

Bilateral vagotomy

Many surveys of the extensive literature on vagotomy have been presented (e.g. 90 158, 168 214 225 246) Most of the experiments were carried out in rabbits, rats, and guinea pigs. In dogs and cats on the contrary vagotomy permits indefinite survival (214) The following short survey of the literature will serve only as a background to my own experiments reported in this section. The account refers to vagotomy without previous overhydration and vagotomy in overhydrated animals.

a) *Vagotomy* After bilateral vagotomy in experimental animals, Lower (1664) noted death from dyspnoea, Vieussens (1715) accumulations of blood in the lungs, and Valsalva (1740) blood-stained f oth in the mouth. Haller (1756—1760) ascribed the pulmonary changes to aspiration of gastric contents. Legallois (1812) suggested that the cause of death would be asphyxia due to a reduced glottis, hyperaemia in the lungs, and/or the presence of serous fluid in the bronchi. Traube (1846) showed that tracheotomy protected rabbits against agotomy oedema, concluding that ocal-cord paraly i was the mechanism of the lung lesion. Schiff (1840) in contradiction of Traube, found that pulmonary oedema occurred even after tracheotomy and did not note any lung lesion fter bilateral division of the recurrent laryngeal nerve. Schiff formed the hypothesis f pulmonary vasomotor paraly is whereas Claude Bernard (1858) ascribed the lesions to altered respiratory dynamics Löwit (1893) did not find any rise in left atrial pressure and concluded that the lung oedema was brought about by vasodilatation with increased accumulation of blood and increased vascular permeability Shafer (1920) noted pulmonary oedema in association with slow asphyxia without vagotomy and suggested that this was the causative f ct in the lung oedema following vagotomy Farber (1937) found that tracheotomy and artificial respiration did not protect rabbits against vagotomy oedema. On direct inspection he noted that the lung lesions began s petechia which gradually fused into large areas showing red discoloration which t topsy were found to be consolidated areas. He considered that vagal denervation increased the permeability of the lung capillaries. Lorber (1939) found that with careful bronchial toilet after vagotomy outspoken lung oedema did not occur He suggested that circulatory failure in association with anoxia could be a factor in olved in vagotomy oedema.

Short (1944) showed that tracheotomy lone without vagot my can give rise to tagnation of secretion in the bronchi and cause slight pulmonary oedema. Reichsman (1946) found that the inspiratory phase wa prolonged after vagotomy and that even with other techniques impeded inspiration can cause pulmonary oedema. This was confirmed in experiments by

Baruch (1935) who found that oedema developed in the hilar and the basal regions in dogs submitted to slight inspiratory resistance to breathing for 6 hours Reichsman found as did Farber (1940) that the blood and plasma volumes decreased greatly after vagotomy Haddy et al (1950) noted that the pulmonary vein pressure rose as a result of inspiratory air way resistance. Keyrilâinen & Paasonen (1958) showed that ether anaesthesia at vagotomy in rats reduced the survival time in comparison with chloral, urethane, and sodium pentobarbitone anaesthesia, that atropine in high doses reduced the degree of pulmonary oedema that sex and age was of no significance in the formation of pulmonary oedema and adrenalectomy had no effect on its development.

b) *Vagotomy—overhydration.* Kraus (1913) found that rapid intravenous infusion of saline, about 400 ml before and 50 ml after the vagotomy produced frothy pulmonary oedema in rabbits If adrenaline was given in addition the oedema was aggravated Kraus considered that the lung oedema was due to loss of innervation of the lung vessels. Brunn (1913) reported that tracheotomized vagotomized rabbbits developed pulmonary oedema less readily than did non tracheotomized rabbits after the same amount of saline. If the rabbits were nephrectomized bilaterally before the vagotomy and the infusion, pulmonary oedema did not occur Farber (1940) however noted no difference in the frequency of pulmonary oedema (gross observations) in association with overhydration between tracheotomized and non tracheotomized vagotomized rabbits. After unilateral vagotomy pulmonary oedema rarely occurred in the rabbits, despite infusion of saline at a rate of 40 ml per minute the total amount being 250—400 ml Reichsman (1946) showed that pulmonary oedema could be precipitated regularly by means of rapid intravenous infusion of saline in rats submitted to inspiratory resistance to breathing Unilateral or bilateral sympathectomy reduced the tendency toward pulmonary oedema following massive infusions of saline in vagotomized dogs (52)

Own investigations

The purpose of this series was to study the frequency of pulmonary oedema at different degrees of uraemia in non overhydrated and overhydrated vagotomized rabbits whose ureters had been tied bilaterally

All the animals were first submitted to bilateral ureteral ligation under local anaesthesia. In the overhydrated series the animals were given fluid before they underwent vagotomy Bilateral vagotomy 0—48 hours after the ureteral ligation was performed under local anaesthesia through a median incision in the neck.

29 non-overhydrated anuric rabbits were divided into two series which were vagotomized a) 0—4 hours (15 animals) and b) 48 hours (14 animals) after the ureteral ligation

28 overhydrated anuric rabbits were divided into two series which were vagotomized a) 3—5 hours (14 animals) and b) 48 hours (14 animals) after the ureteral ligation. Two hours before the vagotomy Ringer's solution was infused intravenously for 1 1/2 hours in amounts equalling 15 % of the body weights

Two rabbits which died from suffocation during the operation were excluded from the series 2 rabbits which died within 5 minutes of the vagotomy are included Three rabbits which received infusion after the vagotomy were markedly distressed by even the

lightest possible fixation and died from suffocation during the infusion.

Respiratory rate measurements and electrocardiograms were taken before the ureteral ligation, before and after the vagotomy and before the blood letting whereas chest X ray could not be performed as planned, because the animals had attacks of suffocation when being fixed on the table. Four of those that were examined first died during the examination. Radiographs were therefore taken only before the bloodletting or within 5 minutes of spontaneous death. In view of the risk of suffocation, the animals were allowed to move freely in the room after the vagotomy

The rabbits that did not die spontaneously were bled within 14 /2 hours of the vagotomy Four moribund rabbits were bled within 5—10 hours. In spite of frequent checkings even during the night, it was difficult to estimate the probable survival time, that is, the suitable time for bloodletting before the planned time Four rabbits were excluded form the series, as rigor mortis had already set in when death was noted.

Survival time

The series of non-overhydrated anuric rabbits a) Among 16 animals which had been anuric for 0—4 hours before the vagotomy none died during the first 7 hours, 2 died spontaneously within 11 hours 13 were bled within about 14 / hours. b) Out of 17 animals which were vagotomized after 48 hours of anuria, 4 died spontaneously within the first 7 hours and another 5 within about 14 1/2 hours 4 were bled in moribund states within 5—10 hours of the vagotomy and 4 survived long enough to be bled with in about 14 1/2 hours.

The series of overhydrated anuric rabbits a) Out of 15 animals which had been anuric for 3—5 hours, 4 died spontaneously within the first 7 hours and another 6 within 14 hours of the vagotomy 5 were bled within about 14 1/2 hours. b) Out of 14 animals which were vagotomized after 48 hours of anuria, 7 died spontaneously within the first 7 hours and a further 3 within 14 hours 4 were bled within about 14 1/2 hours.

In these groups the average survival time was lowest for overhydrated animals with anuria for 48 hours (8.2 hours) and highest for non-overhydrated animals with anuria for 0—4 hours (13.8 hours)

Symptoms in vivo

Under this heading a short account will be given of the generalized symptoms that were noted in vivo after vagotomy It will be seen from the account of the postmortem findings under the next heading that the diagnosis of lung oedema by the criteria that were well applicable in the previous series offered great difficulties in some of the vagotomy cases. Only after a detailed discussion of the sources of error in the different criteria under the experimental conditions used here, will it be possible to discuss the relation between pulmonary oedema, diagnosed with varying degrees of reliability

and symptoms in vivo This will be done under a separate heading below

Appearance, behaviour and respiration As is usual vagotomy was regularly followed by bradypnoea In about two-thirds of the animals respiration became piping and rattling The respiratory difficulties increased on slightest exertion and particularly on attempts at holding the animals fast Stridulous breathing was commoner in overhydrated than in non-overhydrated animals The general condition was relatively little affected by the vagotomy

The respiratory rate fell in the whole series from an average of 137 (60—202) per minute before the vagotomy to 51 (24—84) per minute after the operation.

E C G and heart rate The heart rate increased from an average of 230 (174—316) beats per minute before the vagotomy to 263 (180—340) beats per minute immediately after the vagotomy

Among 31 rabbits with anuria for *48 hours E C.G before vagotomy* showed no abnormalities in 20 absence of T waves in 8 negative T waves in 2 and a deformed QRS-complex in 1 A few minutes after the vagotomy the E C.G was normal in 10 of these rabbits the T wave absent in 2 and negative in 8 and the QRS-complex deformed in 11 Out of 30 animals with anuria for only a few hours 2 had slight E.C.G changes after the vagotomy

The heart shadow increased within a few minutes of the vagotomy in 6 of 10 examined non-overhydrated animals Subsequent assessment was difficult because of pulmonary changes (Tables 24 and 25)

Chest X ray showed no abnormalities in 10 cases examined before and immediately after the vagotomy

Radiographic pulmonary changes postmortem findings and pulmonary oedema

As already mentioned the diagnosis of pulmonary oedema in the vagotomy series offered great difficulties in some cases the uncertainty attached to each criterion will be discussed in the following It will be seen from Tables 24 and 25 that this refers only to some of the 57 animals. 25 rabbits (44 %) had, at most, two of six criteria of pulmonary oedema and hence pulmonary oedema was regarded as not present. In 18 cases (31 %) the presence of pulmonary oedema was very probable 8 rabbits had all the six criteria and 7 had five criteria In 3 rabbits with four criteria (lung weights *19 02 g 11 12 g and 9.58 g*) but without or with insignificant pneumonia microscopically X ray was not performed but would probably have supported the diagnosis of pulmonary oedema In the remaining 14 (25 %) the presence of pulmonary oedema was more difficult to establish or to exclude

As has been mentioned earlier X ray evidence of unilateral or apical changes was seen in 11 of 31 animals with lung lesions The most frequent microscopical finding in these 11 cases was pneumonia with atelectatic and

	No.		(g/kg body weight)	In lung %	by gross observation	in the air-way		changes	[illegible] mental				[illegible] hours		
I. V. potency 0—4 hours after ligation of ureters	1	L	11.36	84.6	+++	+++	++	+++	?	4	0	87	10.4	S	
	2	L	7.86	87.7	+++	+++	++	++	+	4	3	80	7.0	S	p
	3		7.60	81.9	+	0	0	+++?	?	0	0	115	14.7		p
	4		6.21	82.4	+	++	0	++	0	0	0	107	14.8		p
	5		5.66	82.9	0	0	0	—	—	0	0	110	14.5		
	6		5.07	81.1	0	0	—	0	0	0	0	124	14.7		
	7		4.97	79.1	0	0	—	0	0	0	3	70	14.6		
	8		4.83	77.9	0	0	—	0	0	2	0	90	14.6		
	9		4.71	82.2	0	0	0	0	++	4	2	86	14.3		
	10		4.68	81.7	+	0	0	++	0	0	3	93	14.5		
	11		4.59	81.6	0	0	—	0	0	0	0	133	14.6		
	12		4.49	82.2	0	0	0	0	++	3	0	110	14.4		
	13		4.46	81.8	0	0	+	0	+	1	0	108	14.8		
	14		4.33	82.3	0	0	—	0	0	0	3	96	14.6		
	15		4.23	83.8	0	0	—	0	0	0	0	121	14.0		
Mean			5.63	83.6						1	1	102			
II. V. potency 48 hours after ligation of ureters	16	L	18.42	80.9	+++	++	++	+++	?	5	5	—	6.0	S	p
	17	I	10.93	83.5	++	+	++	+++?	?	0	3	275	7.6		p
	18	L	10.38	83.3	+++	+++	+	+	+	0	3	—	2.2	S	
	19	L	8.07	81.9	+	0	+	++?	?	0	0	253	5.0		
	20		8.68	82.4	+	0	0	+++?	?	1	0	[illegible]	14.3	S	p
	21	L	8.50	81.5	+	0	++	++?	+	3	3	261	7.0	S	
	22		6.18	83.2	+	0	0	+++?	?	0	2	225	14.0		
	23		5.48	82.7	0	0	—	+++?	0	0	0	258	6.1		
	24		4.91	81.7	+	0	0	+++?	+	0	2	216	10.2		
	25		4.76	80.9	0	++	0	0	+	2	6	279	6.0	S	
	26		4.34	83.0	0	0	0	0	++	5	9	228	14.3		
	27		4.33	81.9	0	0	—	0	0	0	0	218	14.5		
	28		3.78	82.5	0	0	0	0	++	11	10	170	0.0	S	
	29		3.63	80.3	0	0	—	0	0	0	0	227	14.8		
Mean			7.38	83.3						3	5	211			

Table 25 Results after infusion of Ringer's solution, 15 % of the body-weight for 1 1/2 hours and bilateral vagotomy in anuric rabbits whose ureters had been tied 3—5 and 48 hours respectively before the vagotomy
L = at least three criteria of pulmonary oedema. P = microscopical evidence of severe pneumonia. S = spontaneous death

Group	Rabbit No.		Lung weight g/kg body weight	Water content of lung %	Pulmonary oedema by gross observation	Frothy fluid in the airways	Alveolar oedema	X-ray: Lung changes	X-ray: Cardiac enlargement	Pleural fluid ml	Ascites ml	N.P.N. mg per 100 ml	Length of survival after vagotomy hours		
I Vagotomy 3—5 hours after ligation of ureters	30	L	10.02	90.0	++	++	0	—	—	14	4	100	13.5	S	
	31	L	13.01	87.1	++	0	+	+++	?	18	2	103	13.9	S	
	32	L	12.82	87.1	+++	++	++	—	—	38	20	110	13.1	S	P
	33	L	11.72	87.7	+++	++	+	++	+++	21	4	90	11.4		
	34	L	10.42	89.4	++	+	+	+	++	1	20	103	14.8		
	35	L	9.58	87.3	++	++	0	—	—	8	10	47	3.2	S	
	36	L	9.18	85.7	++	+++	0	+++	?	3	38	90	7.0	S	
	37	L	7.80	83.7	+	++	0	+++	?	20	7	97	11.0		P
	38	L	7.09	86.1	++	0	+	++	++	6	12	97	5.0	S	
	39		7.05	81.0	0	0	0	++	?	2	8	44	0.3	S	
	40		5.17	82.5	0	0	0	+++	?	60	1	81	13.7	S	
	41		5.38	84.7	0	0	—	+	+	11	2	78	14.5		
	42		4.88	81.7	++	0	0	++	?	1	5	50	0.5	S	
	43		4.50	82.0	0	0	—	+++	?	34	5	72	11.3		
	Mean		9.14	[illegible]						17	10	80			
II Vagotomy 48 hours after ligation of ureters	44	L	11.72	90.2	+++	+	0	+++	?	20	55	158	4.3	S	
	45	L	13.01	90.4	+++	+++	+	++	?	2	45	238	3.4	S	
	46	L	12.11	86.8	+++	++	++	—	—	9	385	310	0.8	S	
	47	L	11.29	81.1	+	0	+++	—	—	3	384	210	13.1	S	
	48	L	11.12	83.1	+++	++	+++	—	—	3	288	187	11.0	S	
	49	L	7.37	81.9	+	0	0	++?	0	4	0	220	0.7	S	
	50		7.13	83.0	+	0	0	—	—	2	2	—	0.1	S	
	51	L	9.12	83.2	++	0	+	+++	?	36	147	180	2.8	S	
	52		5.98	82.1	+	0	0	++?	?	0	230	221	14.1		P
	53		61	81.2	++	0	+	—	—	0	35	180	0.1	S	
	54		5.57	81.7	+	0	0	+++	?	5	300	102	14.4		P
	55		4.80	82.1	+	0	0	++?	0	30	164	212	11.5		
	56		4.07	82.0	+	++	0	++?	?	41	281	211	11.1		P
	57		4.21	81.1	0	0	—	+++	?	[illegible]	[illegible]	[illegible]	[illegible]		
	Mean		[illegible]	[illegible]											

haemorrhagic areas. None of these rabbits had a sufficient number of the criteria required for the diagnosis of pulmonary oedema in this series, that is, three criteria including increased water content or microscopical alveolar oedema (see the next heading)

Bilateral diffuse lesions in all the lobes were seen in 23 of the 34 cases. Radiographic pulmonary changes were seen in all animals that had sufficient criteria of pulmonary oedema. In 6 of the 23 animals with bilateral changes, the number of criteria required in this series was not sufficient, however microscopically pneumonia, atelectasis, and/or haemorrhages were present.

Five rabbits, which at autopsy were found to have atelectasis as the main finding by gross and microscopical examination, normal lung weights, and more than 30 ml of pleural fluid (4 cases) had radiographic pulmonary changes which in 2 cases were bilateral and diffuse, in 2 cases unilateral and in 1 case localized in the upper lobes.

The radiologist has in 13 cases (see the sign ?" in Tables 24 and 25) stated the presence of pulmonary processes other than oedema or both pulmonary oedema and some other process. None of these rabbits had microscopical evidence of pulmonary oedema alone.

Gross observation at autopsy. The average discharge of blood at the bloodletting wa 49 (28—80) ml as against 64 (30—110) ml in the series of rabbits submitted to ureteral ligation without vagotomy A reduced amount of blood after vagotomy was also noted by Reichsman and Farber (84 214) The amounts of ascitic fluid and pleural fluid (Tables 24 and 25) differed insignificantly from those found in the animals of the series described in previous chapters.

Gross evidence that could indicate pulmonary oedema was present in 30 out of 57 rabbits. All the animals with pulmonary oedema evidenced by the criteria used in this series had such macroscopical changes. In 13 animals (Diagram 18) with such changes the diagnosis of pulmonary oedema could not be established by the criteria used in this series.

The macroscopical picture varied Mottled or wholly dark red lung surfaces (+++) were noted in 10 rabbits (the other criteria were also positive in 8 1 criterion was lacking in 2) 10 rabbits had spotted, streaky or partly mottled lung surfaces (++") but microscopical examination showed that one whitish-purple lung (no 42) was atelectatic hyperaemic and that in one brownish-pink spotted lung (no 17) there were large areas of pneumonia.

16 animals had changes graded as + a frequent finding in this group was brown, even and well-defined lesions which seemed to have arisen in the upper lobes. The observed changes ranged from single spots in the upper lobes to brown areas extending over the upper and middle lobes and the upper parts of the lower lobes. The rest of the lung surface was in some cases blue and pink as well, and in others also covered with red dots or with spots. Only those changes that extended beyond the upper lobes were

Table 25 *Results after infusion of Ringer's solution 15 % of the body weight for 1 1/2 hours and bilateral vagotomy in anuric rabbits whose ureters had been tied 3—5 and 48 hours respectively before the vagotomy L=at least three criteria of pulmonary oedema. P=microscopical evidence of severe pneumonia S= spontaneous death*

Group	Rabbit No.		Lung weight g/kg body weight	Water content in lung %	Pulmonary oedema by gross observation	Frothy fluid in the airways	Alveolar oedema	X-ray Lungs changes	X-ray Cardiac enlargement	Pleural fluid ml	Ascites ml	N P N mg per 100 ml	Length of survival after vagotomy hours		
I. Vagotomy 3—5 hours after ligation of ureters	30	L	10.02	90.6	++	++	0	—	—	14	4	100	13.5	S	
	31	L	13.01	87.1	++	0	+	+++	?	18	2	103	13.0	S	
	32	L	12.82	87.1	+++	++	++	—	—	38	20	110	13.1	S	P
	33	L	11.72	87.7	+++	++	+	++	+++	21	4	90	11.4		
	34	L	10.42	80.4	++	+	+	+	++	1	20	103	14.8		
	35	L	9.58	87.3	++	++	0	—	—	8	10	47	3.2	S	
	36	L	9.18	85.7	++	+++	0	+++	?	3	38	66	7.9	S	
	37	L	7.80	83.7	+	++	0	+++	?	20	7	97	14.0		P
	38	L	7.03	86.4	++	0	+	++	++	0	12	67	3.0	S	
	39		7.05	84.0	0	0	0	++	?	2	8	11	0.3	S	
	40		5.17	82.5	0	0	0	+++	?	66	1	81	13.7	S	
	41		5.38	84.7	0	0	—	+	+	11	2	78	14.5		
	42		4.88	84.7	++	0	0	++	?	1	5	60	0.5	S	
	43		4.50	82.0	0	0	—	+++	?	34	5	72	14.3		
	Mean		9.14	85.9						17	10	80			
II. Vagotomy 48 hours after ligation of ureters	44	L	11.72	90.2	+++	+	0	+++	?	20	55	158	4.1	S	
	45	L	13.01	90.4	+++	+++	+	++	?	2	45	238	3.4	S	
	46	L	12.11	86.8	+++	++	++	—	—	0	385	210	0.8	S	
	47	L	11.20	81.1	+	0	+++	—	—	3	384	216	13.1	S	
	48	L	11.12	83.4	+++	++	+++	—	—	3	288	187	11.0	S	
	49	L	7.37	81.9	+	0	0	++?	0	4	70	220	0.7	S	
	50		7.13	83.9	+	0	0	—	—	2	2	—	0.1	S	
	51	L	6.12	88.2	++	0	+	+++	?	30	147	180	2.8	S	
	52		5.98	82.1	+	0	0	++?	?	0	230	221	14.4		P
	53		5.61	81.2	++	0	+	—	—	0	35	180	0.1	S	
	54		5.57	81.7	+	0	0	+++	?	5	360	192	14.4		P
	55		4.80	82.1	+	0	0	++?	0	30	164	212	11.5		
	56		4.60	82.0	+	++	0	++?	?	41	281	244	11.4		P
	57		4.21	81.1	0	0	—	+++	?	4	3	161	2.4	S	
	Mean		8.21	84.0											

haemorrhagic areas. None of these rabbits had a sufficient number of the criteria required for the diagnosis of pulmonary oedema in this series, that is, three criteria including increased water content or microscopical alveolar oedema (see the next heading)

Bilateral diffuse lesions in all the lobes were seen in 23 of the 34 cases. Radiographic pulmonary changes were seen in all animals that had sufficient criteria of pulmonary oedema. In 6 of the 23 animals with bilateral changes, the number of criteria required in this series was not sufficient however microscopically pneumonia, atelectasis, and/or haemorrhages were present.

Five rabbits, which at autopsy were found to have atelectasis as the main finding by gross and microscopical examination, normal lung weights, and more than 30 ml of pleural fluid (4 cases) had radiographic pulmonary changes which in 2 cases were bilateral and diffuse, in 2 cases unilateral, and in 1 case localized in the upper lobes.

The radiologist has in 13 cases (see the sign ?" in Tables 24 and 25) stated the presence of pulmonary processes other than oedema, or both pulmonary oedema and some other process. None of these rabbits had microscopical evidence of pulmonary oedema alone.

Gross observation at autopsy The average discharge of blood at the bloodletting was 49 (28—80) ml as against 64 (30—110) ml in the series of rabbits submitted to ureteral ligation without vagotomy A reduced amount of blood after vagotomy was also noted by Reichsman and Farber (84 214) The amounts of ascitic fluid and pleural fluid (Tables 24 and 25) differed insignificantly from those found in the animals of the series described in previous chapters.

Gross evidence that could indicate pulmonary oedema was present in 38 out of 57 rabbits. All the animals with pulmonary oedema evidenced by the criteria used in this series had such macroscopical changes. In 13 animals (Diagram 18) with such changes the diagnosis of pulmonary oedema could not be established by the criteria used in this series.

The macroscopical picture varied Mottled or wholly dark red lung surfaces (+++) were noted in 10 rabbits (the other criteria were also positive in 8 1 criterion was lacking in 2) 10 rabbits had spotted, streaky or partly mottled lung surfaces (++") but microscopical examination showed that one whitish purple lung (no 42) was atelectatic hyperaemic and that in one brownish pink spotted lung (no 17) there were large areas of pneumonia.

16 animals had changes graded as + a frequent finding in this group was brown, even and well-defined lesions which seemed to have arisen in the upper lobes. The observed changes ranged from single spots in the upper lobes to brown areas extending over the upper and middle lobes and the upper parts of the lower lobes. The rest of the lung surface was in some cases blue and pink as well, and in others also covered with red dots or with spots. Only those changes that extended beyond the upper lobes were

graded as "+ Throughout this group the diagnosis of pulmonary oedema was in doubt

Frothy fluid in the air ways was noted in 10 of the 57 animals. Because of the profuse amounts of viscid mucus and fluid in the mouth nose and trachea in vagotomized rabbits, notably overhydrated ones, the diagnosis of pulmonary oedema by the presence of frothy fluid in the air passages was doubtful in this series Phlegm or aspirated fluid can form froth and oedema fluid may contain very little air and resemble phlegm

In 16 of the 19 animals with frothy fluid there were a sufficient number of the criteria of pulmonary oedema required in this series frothy fluid was present in the majority of the rabbits that had the most distinct evidence of pulmonary oedema Exceptions were 2 animals with seemingly considerable pulmonary oedema (lung weights 13 01 and 11 29 g per kg body weight no pneumonia microscopically) Three rabbits (lung weights 6 21 4 76 and 4 69 g) had frothy fluid in the trachea but bronchitis—bronchopneumonia were diagnosed microscopically and water contents were low according to the criteria used in this series pulmonary oedema was regarded as not present

Thus the presence of frothy fluid cannot be taken as conclusive evidence of pulmonary oedema in vagotomized animals

The lung weight exceeded the upper limit of normal range in 31 out of 57 animals 22 of these 31 rabbits had criteria of the kind required for the diagnosis of pulmonary oedema in this series (see the following) Poulsen (206) stated that pneumonia and hyperaemia lead to increased lung weight This may be the explanation of the increased lung weights in the 9 rabbits which did not have pulmonary oedema by the criteria used in this series microscopically these 9 rabbits had pneumonia or hyperaemia but no alveolar oedema This assumption is further supported by the data in Diagrams 14 and 16 in which the pneumonia cases from all the series and 3 rabbits without ureteral ligation have been brought together

Under the experimental conditions of this study the lung weight is thus of definite value only in excluding higher degrees of pulmonary oedema and when pneumonia and hyperaemia can be ruled out

The water content exceeded the upper limit of normal range in 21 out of the 57 animals (15 of these 21 were overhydrated) In 17 of the 21 animals with increased water contents the number of other criteria used in this series was sufficient to establish the diagnosis of pulmonary oedema (Tables 24 and 25) 4 animals had normal water contents, but pulmonary oedema by the criteria used in this series they had gross evidence of pulmonary oedema increased lung weights, and microscopical evidence of alveolar oedema but also appreciable haemorrhages (2 cases) or hyperaemia (2 cases) in 1 of the latter profuse stringy exudate was seen

If the water content in the lung is only slightly increased in overhydrated

cases and if pulmonary changes other than oedema co-exist, the water content is of uncertain value in the diagnosis of pulmonary oedema.

Microscopical examination showed in the vagotomy series pneumonia, atelectasis, haemorrhage, hyperaemia and/or oedema in varying combinations. When one process predominated, the microscopical diagnosis was easy but if several processes co-existed, evaluation became a problem. Was oedema secondary to haemorrhages or small pneumonic lesions, or were there both pulmonary oedema and pneumonia or pulmonary oedema and haemorrhage?

The microscopical appearance was particularly variegated in specimens taken from brown areas (macroscopically +) of lungs from the 16 rabbits with such changes. Four types of microscopical abnormalities were seen in 16 animals: a) the bronchi filled with viscid mucus, leucocytes, and, occasionally foreign matter; areas of purulent bronchitis and, in some cases, *peribronchitis*, pneumonic foci; b) atelectasis and emphysema; c) focal haemorrhage and/or blood diffusely in the alveoli. In addition there was d) alveolar oedema of varying extent. All the components were in some cases seen mixed together. In others one of them predominated. Pneumonic areas were seen in 8 and alveolar oedema graded as + in 3 of these 16 rabbits.

Th series comprised 87 animals. Alveolar oedema was seen in 19 out of 45 examined cases. 12 animals with normal lung weights, no frothy fluid, and gross evidence of pulmonary oedema were not examined microscopically (one of them, however, was found to have a slightly increased water content and slight (+) radiographic pulmonary changes). In 17 of 19 cases with alveolar oedema the number of other positive criteria required in this series was sufficient to establish the diagnosis of pulmonary oedema. Exceptions were 2 animals without pulmonary oedema by the criteria used here which had alveolar oedema; one of them had a water content of 84.8 % and white spots locally on the lung surface, (focal oedema?). The other one had hyperaemia and a spotty lung surface (++ macroscopically); unfortunately radiography was not performed, but as the lung weight and water content were normal, only insignificant pulmonary oedema can have been present. Six animals with pulmonary oedema evidenced by all the other criteria used in this series (except for frothy fluid in 1 case) did not have alveolar oedema, all being overhydrated. The absence of alveolar oedema in overhydrated rabbits with high water contents and probable lung oedema was noted earlier in the dextran and adrenaline series. The difficulty of visualizing alveolar oedema in such cases has been described by Visscher et al. (240)

Pneumonia or bronchitis was seen microscopically in 11 of 28 rabbits with anuria for 48 hours and in 6 of 29 with anuria for a few hours. Seven out of 28 overhydrated rabbits and 10 out of 29 non-overhydrated rabbits had such infection. Seven out of 17 rabbits with pneumonia and bronchitis were

also judged as having pulmonary oedema. Bronchiolitis was not noted in any of the cases and periarterial oedema was seen in only 1 case.

Thus the microscopical picture was in this series of great value, particularly in the differential diagnosis. The disadvantage of the method was the difficulty of visualizing protein poor alveolar oedema and to exclude or establish the presence of pulmonary oedema in cases of co-existent alveolar oedema and pneumonia atelectasis, haemorrhage, or hyperaemia. In cases of such mixed pulmonary processes the diagnosis of pulmonary oedema was uncertain even when the alveolar oedema was relatively extensive and other criteria were desirable to support the diagnosis.

Pulmonary oedema and symptoms in vivo

The appearance and behaviour of the animals gave no aid in determining the presence of pulmonary oedema pneumonia stagnation of secretion, or atelectasis but in animals that moved about relatively easily no very severe changes of the this kind were seen at autopsy

In 34 rabbits which later on did not exhibit pulmonary oedema the respiratory rate averaged 52 (24—84) per minute and in 23 rabbits which later on exhibited pulmonary oedema according to the criteria used in this series, it averaged 50 (24—72) per minute. Bradypnoea persisted even in rabbits that died with all the other criteria indicating pulmonary oedema

Thus, the usual clinical symptoms of pulmonary oedema—tachypnoea and dyspnoea—could not be used as criteria for the diagnosis in the vagotomy series

E.C.G and heart rate Immediately after the vagotomy the heart rate in 23 rabbits which later on exhibited pulmonary oedema by the criteria used here averaged 267 (187—340) beats per minute and in 34 rabbits without subsequently established pulmonary oedema it average 260 (180—308) beats per minute. At the bloodletting the average heart rate was in 5 rabbits with sufficient criteria of pulmonary oedema 248 (214—294) and in 26 rabbits without sufficient criteria of pulmonary oedema 266 (182—321) beats per minute.

Six out of 21 animals with E.C.G disturbances eventually developed pulmonary oedema according to the criteria used here. Six of 19 rabbits in which pulmonary oedema was later on established by the criteria used here, had severe E.C.G changes immediately after the vagotomy

Thus, the heart rate and E.C.G gave no guidance to the diagnosis of pulmonary oedema or the prognostication of its development.

The heart shadow was enlarged in 6 rabbits with pulmonary oedema and of normal size in 1 rabbit.

Criteria of pulmonary oedema

The diagnosis of pulmonary oedema was difficult to establish in cases of pulmonary changes characterized by concurrent pneumonia atelectasis,

haemorrhage, hyperaemia, and oedema. In this series it was particularly difficult to distinguish rabbits with pulmonary oedema from rabbits without pulmonary oedema. According to the rule that at least three criteria for the diagnosis of pulmonary oedema must be satisfied 30 out of 57 rabbits had pulmonary oedema 4 of 15 slightly uraemic non-overhydrated, 7 of 14 severely uraemic non-overhydrated, 9 of 14 slightly uraemic overhydrated, and 10 of 14 severely uraemic overhydrated rabbits.

However at more critical examination this method for establishing the diagnosis of pulmonary oedema proved unsatisfactory in this series. It was found that 7 rabbits (nos. 3 4 20 22 52, 54 and 56) certainly had three positive criteria of pulmonary oedema (increased lung weight, macroscopical and microscopical pictures that could be compatible with pulmonary oedema, and/or in 2 cases, frothy fluid in the air ways) but proved to be cases of outspoken pneumonia Microscopical examination showed pneumonic areas but no alveolar oedema the changes were predominantly localized in the upper lobes or unilaterally and the water content was low One of these rabbits had even four positive criteria of pulmonary oedema, but the water content was normal and there was no alveolar oedema.

With the requirement that at least five criteria for the diagnosis of pulmonary oedema should be satisfied, 15 cases would have been diagnosed as pulmonary oedema 2 slightly uraemic non-overhydrated 3 severely uraemic non-overhydrated, 7 slightly uraemic overhydrated, and 3 severely uraemic overhydrated. On the other hand 4 rabbits with four positive criteria but in which X ray was not performed, would not have been included under this diagnosis, nor would rabbits with slight pulmonary oedema.

Other methods of assessment were therefore tried. The microscopical picture and the water content seem, according to Poulsen (206) and Diagrams 14—17 to provide the best aid in the differentiation between pneumonia and pulmonary oedema *In this series three positive criteria were required for the diagnosis of pulmonary oedema but a water content exceeding the upper limit of normal range or alveolar oedema established microscopically had to be one of them.* An account of the result obtained by this method will be given below under the next heading.

This method, too is not wholly satisfactory The microscopical picture and the water content are in some situations misleading and the fulfilment of both criteria cannot be required, because at a very high water content visible alveolar oedema can be absent. Moreover as will be seen from the account of symptoms in vivo and post mortem findings, all the criteria used in this vagotomy series are uncertain in some situations. For this reason attempts at making a diagnosis of pulmonary oedema on the basis of various combinations of these *criteria* can lead to false results. The diagnosis of pulmonary oedema *based on the criteria* used here was sometimes in doubt.

a) Pulmonary oedema According to the criteria used here, 23 rabbits had *pulmonary* oedema. The diagnosis was in 8 cases (35 %) supported by all the six criteria in 7 cases (30 %) by five, *in 7 cases (30 %) by 4 and in 1 case* (5 %) by three criteria Six rabbits were not examined by X ray 2 with *five criteria*, 3 with *four and* 1 with three criteria of pulmonary oedema all of them had such marked macroscopical changes that X ray evidence would in all probability have been ob tained if the examination had been performed.

The diagnosis was most uncertain in *5 animals in which four* out of six or three out of five criteria were positive (nos. 10 21 47 49 and 51) None of *these had pneumonia microscopically* but nos. 21 and 51 had moderate hyperaemia besides alveolar oedema no 19 had haemorrhagic alveolar oedema and in no 49 the picture was peculiar in *that the lungs* were pink there was no frothy fluid in the air ways, and the microscopical examina *tion showed no abnormalities, but the* lungs were swollen and did not col lapse the lung weight was 7.37 g per kg body weight, the water content was increased (84 9 %) and the pulmonary changes were *clearly visible* in the radiograph (oedema?)

The diagnosis was also uncertain in the rabbits that seemed to have coexistent pulmonary oedema and pneu monia. A slight increase in the leucocyte count was noted in half the rab bits with pulmonary oedema but foci of particularly numerous cells were seen in only 5 cases nos. 2 16 17 32 and 37 *among which* 4 had six and 1 had five positive criteria of pulmonary oedema (radiographs were missing) In 1 case (no 37) the *water content* was increased, 83 7 % but the animal was overhydrated and the alveolar oedema was modest.

The significance of haemorrhages *and hyperaemia is still* more difficult to evaluate, since they occur to some extent in virtually all animals with *pulmonary oedema*

b) No pulmonary oedema In 34 cases the number of positive criteria was less than three, or both the water con tent and the microscopical picture were normal, and hence pulmonary oedema was not considered to be pres ent 12 of these rabbits had no posi *tive criteria 6 had one criterion*, 9 had two, 6 had three, and 1 had four posi tive criteria. The last mentioned 7 ani mals had pneumonia (see the foregoing) X ray examination was not performed in 3 and *microscopical exa* mination was not carried out in 12 With positive results of the two exa *minations that were not performed* 2 of these 15 cases would have been diagnosed as having pulmonary oede ma according to the criteria establish ed for this series.

One of the rabbits died shortly after the vagotomy the lung weight was 7 13 g per kg body weight and macroscopically the lung surface was spot ted with blue-pink brown there was no *frothy fluid, the water content* was normal and microscopical examina tion showed marked hyperaemia but no alveolar oedema unfortunately the radiograph was a failure.

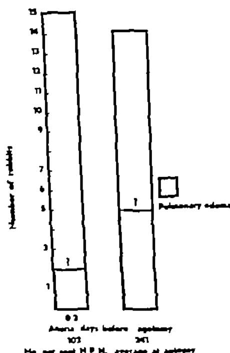


Diagram 12. Frequency of pulmonary oedema after bilateral vagotomy in non-overhydrated rabbits which had been anuric for 0.2 and 2 days, respectively before the operation.

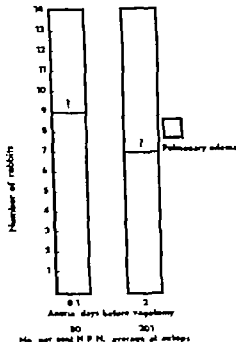


Diagram 13. Frequency of pulmonary oedema after intravenous infusion of Ringer solution, 15 % of the body weight for 1 ½ hours, and bilateral vagotomy in rabbits which had been anuric for 0.1 and 2 days, respectively before the operation.

With the grouping of the rabbits by the criteria used in this series into those with and those without pulmonary oedema, borderline cases were of course inevitable but yet the division seems relatively satisfactory. The interpretation of the results was made difficult, partly by too early death from suffocation (6 out of 57 rabbits died from suffocation within 3 hours of the vagotomy without pulmonary oedema) and partly by the complicated picture that the pulmonary changes presented. In severe cases the diagnosis was easily established. The main difficulty was to exclude milder degrees of lung oedema in the presence of other types of pulmonary changes.

This series shows the difficulties of using the same criteria for the diagnosis in all forms of pulmonary oedema. The experiences gained from this and the other series as regards the range of usefulness of the different methods of examination are discussed in chapter 10.

Pulmonary oedema and uraemia

The series comprised originally 61 animals, but the number was reduced to 57. Three non-overhydrated rabbits

with anuria for 48 hours and 1 overhydrated rabbit with anuria for a few hours were vagotomized but they died and as their dead bodies were not found until after rigor mortis had set in they were excluded from the series

29 non-overhydrated rabbits (Table 24 and Diagram 12) Pulmonary oedema *according to the definition used* here, occurred in 2 of 15 animals which had been anuric for 0—4 hours at the vagotomy and in 5 out of 14 which had been anuric for 48 hours

28 overhydrated rabbits (Table 25 and Diagram 13) Pulmonary oedema *as defined* here occurred in 9 out of 14 animals which had been anuric for 3—5 hours at the vagotomy and in 7 out of 14 which had been anuric for 48 hours.

Thus, *in non-overhydrated animals* the frequency of pulmonary oedema rose with increasing uraemia and in overhydrated animals it fell with increasing uraemia The difference between slightly uraemic and severely uraemic animals within the two series *is not statistically significant however* The above figures for the frequency are uncertain as has been pointed out in the foregoing

Pulmonary oedema and overhydration

The frequency of pulmonary oedema diagnosed by the criteria used here is higher for vagotomized rabbits with electrolyte fluid retention (52 %) than for rabbits without electrolyte fluid retention (24 %) In cases of slight uraemia (64 % and 13 % respectively) the difference could be almost significant ($0.01 < p < 0.02$) The figures for frequency are uncertain however because of the difficulties in establishing the diagnosis of pulmonary oedema in this series.

Survey See page 152

CHAPTER 10

Detection and quantitative study of pulmonary oedema

In this study the main object was to induce slowly supervening pulmonary oedema, in an attempt to imitate clinical conditions. Pulmonary oedema developed mainly in association with four of the methods used, namely after hypervolaemia (blood, dextran) vasoactive drugs (adrenaline) pharmacological agents (barbiturates) and alterations of the peripheral nervous and respiratory systems (vagotomy) The object of using these methods was to try in various ways to throw light on the role of such factors together with uraemia and salt fluid retention in the causation of pulmonary oedema in rabbits with ligated ureters. The experiments were made at different times after the ureteral ligation, that is, at different degrees of uraemia. Half the animals in the adrenaline barbiturate, and vagotomy series were overhydrated with Ringer's solution. The experimental conditions were thus varied for the purpose of evaluating different criteria in the detection and quantitation of pulmonary oedema.

Suitable criteria for the diagnosis of experimentally produced pulmonary oedema have been discussed by Visscher et al. (246) and Poulsen (206) Visscher et al., after a short survey concluded that, except for special purposes, gross observation and lung weight to body or heart weight ratios provide the most reliable practical methods for quantitation of lung oedema, whereas Poulsen recommends determination of lung weight and percentage of water in the lung. In the present experiments ten different methods (all mentioned earlier in the literature) of detecting pulmonary oedema were tried Chest X ray gross observation, observation and collection of frothy fluid in the air ways, measurements of lung weight, estimation of percentage water content in the lung microscopical studies, observation of the appearance and behaviour of the animals, respiratory rate measurements, electrocardiography and determination of lung volume The first six methods, in particular were studied and compared with one another so as to ascertain the conditions under which the methods yield divergent results. A figure for the degree of correlation between the various methods has *not* been given, because this figure depends too much on the nature and degree, etc., of pulmonary oedema chosen (e.g. sudden or slow severe or slight generalized or

focal protein rich or protein poor rich in fluid or rich in blood uncomplicated or complicated with bleeding *pneumonia* hyperaemia atelectasis postmortem changes) Tables 10—25 will give some idea of the degree of correlation between the methods used in this study The criteria of pulmonary oedema found in each experimental series was described in the respective chapters a survey of the observations follows here.

Appearance and behaviour of the rabbits

Acute sudden pulmonary oedema with violent dyspnoea and frothy fluid pouring forth from the mouth and nose, is a well known picture and easily diagnosed. It was only in a few experiments, however that acute severe pulmonary oedema arose (after rapid injection of Narkotal or urea solution intravenously and occasionally after injection of adrenaline intramuscularly) In most experiments the desired slowly supervening pulmonary oedema was obtained.

Slowly supervening pulmonary oedema could be suspected from the animals appearance and behaviour in that they lifted their noses, breathing became distinctly audible, the depth and rate of respiration increased (see the next heading) the nostrils widened and the nose became moist The animals sat still most of the time (particularly striking in the dextran series) except for sudden periods of restlessness (most distinct in the adrenaline series) notably premortally Froth at the nose appeared only as they were dying

At slight degrees of pulmonary oedema these signs could of course be lacking or insignificant. In animals which were ill or weak as a result of uraemia narcosis adrenaline injection or overhydration the presence of *pulmonary oedema could be difficult* to establish. Some of them would, for instance, lie down with irregular and feeble respiration whether pulmonary oedema was present or not.

The differential diagnosis was difficult between dyspnoea and other *forms of pulmonary insufficiency resulting* from for instance extensive atelectasis, profuse pleural effusion or pneumonia Moreover dyspnoea occurred at times in rabbits which had for instance, stagnation of secretion or laryngeal paralysis (the vagotomy series) in animals during and after infusion and in agitated animals (notably in the adrenaline series but even in some normal rabbits) Overhydrated animals had profuse flow of fluid from their noses and in some cases it resembled frothy fluid. Rattling respiration was heard mainly in vagotomized animals indicating stagnation of secretion and air way obstruction similar but milder symptoms were present in overhydrated animals with profuse secretion in the air ways. Bloody flow from the nose was occasionally seen in uraemic animals in the absence of pulmonary oedema. In the adrenaline series there was no correlation between convulsions and the presence of pulmonary oedema.

The observations on the animals

appearance and behaviour were valuable, particularly so in sudden pulmonary oedema, but the assessment became increasingly hazardous the milder the pulmonary oedema or the weaker the animals power of reaction, and the greater the number of differential-diagnostic factors that intercurred. On the other hand, pulmonary oedema could in many cases be excluded by observing the animals, as experimental rabbits which looked and behaved like normal rabbits rarely had pulmonary oedema.

Respiratory rate

The relevant literature contains no studies on the respiratory rate in uraemic rabbits with pulmonary oedema. For rabbits with pulmonary oedema the average respiratory rate was higher than for those without pulmonary oedema in the 'Narkotal' and the adrenaline series but not in the vagotomy series. In the individual case, however the respiratory rate was an unreliable aid to diagnosis, since many animals with pulmonary oedema had a normal or low respiratory rate and since animal with pulmonary processes other than oedema also had a high respiratory rate. The respiratory rate of normal animals in this study averaged 122 (72 –220) per minute. Yet, some restless normal rabbits had a respiratory rate of up to 430 per minute which was higher than the range for rabbits with pulmonary oedema. The highest respiratory rate for those rabbits, 228 (92—380) was noted in the adrenaline series. For rabbits with slight pulmonary oedema in the dextran series the respiratory rate averaged 128 (100—160) per minute.

The respiratory rate fell when the animals were at rest or placed on their backs. It fell with increasing uraemia and after 72 hours of anuria it averaged 79 (33—140) per minute. A respiratory rate lower than normal was noted after infusions, after vagotomy in association with narcosis, and premortally. Highly uraemic rabbits had as a rule, if they developed pulmonary oedema, a high respiratory rate which added to the diagnostic value of the respiratory rate in cases of severe uraemia.

The respiratory rate can be a valuable aid to the diagnosis of pulmonary oedema, when the condition is not complicated by other pulmonary processes and the animals are undisturbed, moribund, anaesthetized, or vagotomized. A normal respiratory rate can be a useful aid in excluding pulmonary oedema.

Electrocardiography and heart rate

Severe electrocardiographic disturbances were very common in these experiments and were due partly to high degree of uraemia (Chapter 8) and partly to other experimental conditions ('Narkotal' narcosis, adrenaline injections, vagotomy infusions). As an aid to the diagnosis of pulmonary oedema E.C.G. was found *to be of* very little value, as most of the rabbits with E.C.G. abnormalities did not have pulmonary oedema, and as many rabbits with pulmonary oedema had only slight E.C.G. changes.

The heart rate proved to be of no

certain diagnostic value, as it changed very little in association with pulmonary oedema and uraemia

The degree of an E C.G disturbance, on the other hand was found to be very useful in estimating the remaining survival time of a uraemic rabbit.

Chest X ray

The only critical evaluation of chest X ray *in experimentally* produced pulmonary oedema has been made by Visscher et al (246) who in quoting Westermarck (252) *state that the* central density ordinarily interpreted as evidence for pulmonary oedema is *almost certainly to be ascribed To a* large extent to distended blood vessels that X ray evidence in some situation *occurs only when the oedema has* reached an advanced stage and that X ray density studies during life is subject to great uncertainty The present author does not share this view on the ground of his own investigations It will be seen from Tables 10—25 and Diagram 18 that in most cases the X ray evidence *is well consistent* with the other criteria, and that chest X ray is one of the most *sensitive methods for evaluation of the oedema* state.

However some difficulties attached *to the method In cases* of very small and of very extensive X ray density changes, in particular it was difficult to make the differential diagnosis between oedema and pneumonia (the vagotomy series) pulm*onary haemor*rhage (*the* dextran and Narkotal series) and atelectasis (the vagotomy and the *adrenaline* series and overhydrated animals) In the series of blood, dextran, and other infusions slight X ray changes, *later unexplained at autopsy* can be suspected to have been ascribable to hyperaemia. On the other hand, profuse pleural *effusion without ate*lectasis gave no X ray changes as has been shown by Alwall & Lunderquist (to be published) The radiographic possibilities of distinguishing between pulmonary oedema on the one hand and pneumonia pulmonary haemorrhage, atelectasis, and hyperaemia on the other have not been brought up for consideration in this work.

The X ray method is uncertain in *cases in which the oedema is localized* mainly at the base of the lung or occurs in the form of numerous circum*scribed lesions (the* Narkotal and the dextran series) X ray was normal in 3 cases of acute sudden pulmonary oedema resulting from rapid injection of Narkotal solution intravenously or adrenaline solution intramuscularly despite the fact that frothy fluid poured forth from the nose and mouth dur*ing the X ray examination*

The number of films that were unsuccessful *because of oblique center*ing or technical mishaps was small Severely ill animals were affected even by very short examination in the hanging position Many of the vagotomized animals could not breath at all in this position. *The lung surface visible in* the radiograph diminished as the animals general condition deteriorated, when there were *profuse* amounts of ascitic fluid which raised the diaphragm and when *the heart was* greatly enlarged all this made the as-

sessment of pulmonary changes difficult in many cases. In uraemic over hydrated rabbits a density in the medial part between the heart and the diaphragm was occasionally seen, but no abnormalities were found at postmortem examination (Lunderquist, to be published)

In animals that appeared to die spontaneously it was not practically possible to take radiographs, as desired, immediately before death. To be able to include a greater number of the spontaneously dead rabbits, films were therefore also taken of the animals in the agonal state or within five minutes of their last breaths. In uncertain cases X ray examination was not made.

At a check up of 18 animals killed by injection of air bloodletting, or injection of 'Narkotal' no X ray density changes in the lungs were seen within five minutes of death. In 2 rabbits, killed by injection of air and anaesthesia respectively no pulmonary changes were seen within two hours but slight changes were noted within four hours of death

Chest X ray is useful in experiments in vivo, when the differential diagnosis presents no difficulties.

Gross observation

According to Visscher et al (240) gross observation and lung weight measurement provide the most reliable methods for quantitation of pulmonary oedema. Farber (83) used ocular inspection to study the development of pulmonary oedema in vagotomized animals initially petechial haemorrhages occurred, thereafter red discoloration, and at autopsy consolidated areas were seen. Poulsen (206) described the lungs in excessive oedema as full with more or less extensive hyperaemia less pronounced pulmonary oedema is not directly visible but must often be suspected in the presence of fairly extensive hyperaemia which usually precedes or accompanies oedema formation.

A comparison between macroscopical evaluation of pulmonary oedema, as graded and defined in this study (Chapter 4) and other criteria of pulmonary oedema of the kind used here has not been found in the relevant literature.

It will be seen from Diagram 18 and Tables 10—25 that gross observation was the most sensitive of the methods used here for diagnosis of pulmonary oedema. In all cases, except 1 there was gross evidence of pulmonary oedema when at least three criteria were fulfilled. It was the best method for detecting minute changes which could be suspected to evidence pulmonary oedema, notably those circumscribed local lesions that were seen most frequently in the 'Narkotal' series.

It will also be seen from Diagram 20 and Tables 10—25 that with the macroscopical evaluation of pulmonary oedema as defined here, pulmonary oedema was diagnosed in *excess* In many cases the gross evidence of slight pulmonary oedema was not supported by other *criteria*. This failure was due to difficulties associated with gradation and the differential diagnosis.

Gradation was difficult, quantita

tively as well as qualitatively in the borderline area between pulmonary oedema and no pulmonary oedema a) the oedema might involve only a small part of the lung mostly the hilar or basal regions b) slight generalized changes of a type that are seen in a more marked form in cases of con siderable oedema led to subjective as sessment, c) other pulmonary processes might co-exist and hence the degree of pulmonary oedema could only be suspected (notably in the vagotomy series)

The differential diagnosis presented problems with respect to atelectasis pneumonia haemorrhages and/or hyperaemia In some cases it was dif ficult to determine whether pulmonary oedema was present and still more difficult, as mentioned above, to esti mate the extent of the oedema macro scopically if several processes coexisted. These difficulties were partic ularly noticeable in the vagotomized animals in animals with hyperaemia or numerous small haemorrhages (the dextran and the adrenaline series) and in the spontaneously dead rabbits with suspected postmortem changes in the lungs.

The macroscopical picture of pul monary oedema was not similar in all the series. Small differences occurred, owing to the co-existence of several pulmonary processes, such as haemor rhages in the dextran series, hyper aemia in the adrenaline series focal changes in the Narkotal series and brown consolidated upper lobe chang es with pneumonia etc., in the vagotomy series. This may necessitate a special method of gradation for dif ferent forms of pulmonary oedema.

Gross observation is useful as a quick method which is expedient in *examining all forms of pulmonary* changes its inadequacy relates to the *differential diagnosis and the* subjec tive gradation.

Frothy fluid in the air ways

Luisada & Sarnoff (170) submitted dogs to tracheotomy and, in their lung oedema studies, noted spontaneous outpouring of frothy sputum in vivo They found that this observation was deceptive in some experiments moderate oedema gave rise to early out pouring of frothy material in others, severe oedema did not They decided that only data based on postmortem findings were to be relied upon in determining the presence and degree of pulmonary oedema In grading the presence of frothy fluid they also em ployed sectioning of the parenchyma and squeezing of the lung The grada tion agreed well with the lung weight

The present author noted enormous amounts of frothy fluid in association with acute pulmonary oedema after injection of adrenaline intramuscular ly or after rapid intravenous injection of urea in animals whose general con dition was good. In some cases, ani *mals in a poor general* condition be cause of for instance severe uraemia died suddenly from severe pulmonary oedema evidenced by all the other criteria with only insignificant amounts of frothy fluid.

As will be seen from Diagram 18 and

Tables 10—25 the presence of frothy fluid in the air ways is in most cases well correlated with the other criteria of pulmonary oedema and frothy fluid occurred preferentially in animals with distinct evidence of pulmonary oedema by the other criteria. The presence of froth was less common than the other five criteria of pulmonary oedema used here

Difficulties attached to the differentiation against aspirated mucus (the vagotomy series) and haemorrhages and secretion in the trachea and bronchi (uraemic and overhydrated animals with profuse tracheal secretion) Luisada & Sarnoff (170) found similar difficulties in vivo in overhydrated dogs. If frothy fluid alone was present in the bronchi and in small amounts, they found it difficult to distinguish between frothy fluid due to pulmonary oedema and frothy fluid that can occur on squeezing of normal lungs at autopsy Frothy fluid in the air ways may possibly form post mortem (Chapter 5) Among 52 animals examined 24 hours after death, 19 % had frothy fluid in the air ways, while no frothy fluid was found in 49 animals which were examined before rigor mortis had set in

In the Narkotal series it was found that severe uraemic animals with fibrinous alveolar oedema at microscopical examination, had no or little frothy fluid in the air ways. Accordingly the composition of the exudate may possibly play a part in the formation of frothy fluid.

Observation of frothy fluid in the air ways, being a simple and quick method, is a valuable aid to the diagnosis of marked pulmonary oedema.

Lung-weight

According to Visscher et al (240) the lung weight together with gross observation, is the most reliable method for diagnosis and quantitation of lung oedema The values found in the present study agree relatively well with those reported by others (Chapter 4) Tables 10— 5 show that the lung weight is generally well correlated with other criteria of pulmonary oedema However the usefulness of the method has some limitations

The lung weight per kg body weight changes noticeably with increasing body weight (Diagram 3) Poulsen (206) has shown that this is also the case in mice It will be seen from the diagram that the experimental animals ought to be of approximately the same weight at the beginning of the experiment To draw the line between animals without and animals with pulmonary oedema on the basis of the lung weight is difficult as will be seen from Courtice's (48) diagram In these experiments, focal pulmonary oedema (occurred mainly in the Narkotal series) caused only slight rises in the lung weight. In the present study the lung weights of normal rabbits were taken as normal values It may possibly have been preferable to use the lung weights of uraemic animals (Tables 7 and 8) as a material for comparison with bled uraemic animals, and the lung weights of animals after infusion of Ringer's solution (Tables 7 12 and 13) as a material for com

tively as well as qualitatively in the borderline area between pulmonary oedema and no pulmonary oedema a) the oedema might involve only a small part of the lung mostly the hilar or basal regions, b) slight generalized changes of a type that are seen in a more marked form in cases of considerable oedema led to subjective assessment, c) other pulmonary processes might co-exist and hence the degree of pulmonary oedema could only be suspected (notably in the vagotomy *series*)

The differential diagnosis presented problems with respect to atelectasis, pneumonia haemorrhages and/or hyperaemia In some cases it was difficult to determine whether pulmonary oedema was present and still more difficult, as mentioned above, to estimate the extent of the oedema macroscopically if several processes co existed. These difficulties were particularly noticeable in the vagotomized animals, in animals with hyperaemia or numerous small haemorrhages (the dextran and the adrenaline series) and in the spontaneously dead rabbits with suspected postmortem changes in the lungs.

The macroscopical picture of pulmonary oedema was not similar in all the series. Small differences occurred owing to the co-existence of several pulmonary processes, such as haemorrhages in the dextran series hyperaemia in the *adrenaline series focal* changes in the Narkotal series and *brown consolidated* upper lobe changes with pneumonia etc., in the vagotomy series This may necessitate a *special method of gradation* for different forms of pulmonary oedema.

Gross observation is useful as a quick method which is expedient in *examining all forms of pulmonary* changes its inadequacy relates to the *differential diagnosis and the subjective* gradation

Frothy fluid in the air ways

Luisada & Sarnoff (170) submitted dogs to tracheotomy and, in their lung oedema studies, noted spontaneous outpouring of frothy sputum in vivo. They found that this observation was deceptive in some experiments moderate oedema gave rise to early out pouring of frothy material in others, severe oedema did not They decided that only data based on postmortem findings were to be relied upon in determining the presence and degree of pulmonary oedema In grading the presence of frothy fluid they also employed sectioning of the parenchyma and squeezing of the lung The gradation agreed well with the lung weight

The present author noted enormous amounts of frothy fluid in association with acute pulmonary oedema after injection of adrenaline *intramuscularly* or after rapid intravenous injection of urea in *animals whose general* condition was good. In some cases ani*mals in a poor general* condition because of for instance severe uraemia *died suddenly from* severe pulmonary oedema evidenced by all the other criteria, with only insignificant amounts of frothy fluid

As will be seen from Diagram 18 and

Tables 10—25 the presence of frothy fluid in the air ways is in most cases well correlated with the other criteria of pulmonary oedema and frothy fluid occurred preferentially in animals with distinct evidence of pulmonary oedema by the other criteria. The presence of froth was less common than the other five criteria of pulmonary oedema used here.

Difficulties attached to the differentiation against aspirated mucus (the vagotomy series) and haemorrhages and secretion in the trachea and bronchi (uraemic and overhydrated animals with profuse tracheal secretion) Luisada & Sarnoff (170) found similar difficulties in vivo in overhydrated dogs. If frothy fluid alone was present in the bronchi and in small amounts, they found it difficult to distinguish between frothy fluid due to pulmonary oedema and frothy fluid that can occur on squeezing of normal lungs at autopsy. Frothy fluid in the air-ways may possibly form post mortem (Chapter 5). Among 62 animals examined 24 hours after death, 19 % had frothy fluid in the air ways, while no frothy fluid was found in 49 animals which were examined before rigor mortis had set in.

In the 'Narkotal' series it was found that severe uraemic animals with fibrinous alveolar oedema at microscopical examination, had no or little frothy fluid in the air ways. Accordingly the composition of the exudate may possibly play a part in the formation of frothy fluid.

Observation of frothy fluid in the air ways, being a simple and quick method, is a valuable aid to the diagnosis of marked pulmonary oedema.

Lung-weight

According to Visscher et al (240) the lung weight together with gross observation, is the most reliable method for diagnosis and quantitation of lung oedema. The values found in the present study agree relatively well with those reported by others (Chapter 4). Tables 10—25 show that the lung weight is generally well correlated with other criteria of pulmonary oedema. However the usefulness of the method has some limitations.

The lung weight per kg body weight changes noticeably with increasing body weight (Diagram 3). Poulsen (200) has shown that this is also the case in mice. It will be seen from the diagram that the experimental animals ought to be of approximately the same weight at the beginning of the experiment. To draw the line between animals without and animals with pulmonary oedema on the basis of the lung weight is difficult, as will be seen from Courtice's (48) diagram. In these experiments, focal pulmonary oedema (occurred mainly in the 'Narkotal' series) caused only slight rises in the lung weight. In the present study the lung weights of normal rabbits were taken as normal values. It may possibly have been preferable to use the lung weights of uraemic animals (Tables 7 and 8) as a material for comparison with bled uraemic animals, and the lung weights of animals after infusion of Ringer's solution (Tables 7, 14, and 18) as a material for com

parison with overhydrated animals Another difficulty attaching to the use of the lung weight in the detection of pulmonary oedema is that poorly bled animals (usually moribund) without pulmonary oedema fall into the same weight-class as well bled animals with slight pulmonary oedema.

The borderline between animals without and animals with pulmonary oedema is still more difficult to establish in spontaneously dead rabbits. Durlacher et al. (73) have shown that the lung weight increases gradually after death the rate of increase depending upon the manner of death. As a material of comparison for the lung weight in non bled animals were used 39 rabbits which died spontaneously in uraemia and were examined within 90 minutes after death These "normal values can of course be only approximate, as the animals died from different causes (overhydration injection of Narkotal and adrenaline, vagotomy uraemia) and were examined at different times (though usually immediately) after death The variation in the time interval between death and examination could not be avoided Continuous night supervision of the animals could not be arranged in these experiments and therefore the times of spontaneous deaths could sometimes only be established approximately but only animals whose bodies were warm and soft when found, have been included in the material (that is those that had been dead for about 1 1/2 hours at the longest) Further investigation into postmortal changes would be valuable. This problem is also of great importance with respect to autopsies in man. Bloodletting after death before any postmortal pulmonary oedema has developed, would provide a possibility to study the problem more closely

Durlacher et al. found that the quantity of oedema present 3 hours after death varies with the method of sacrifice, the route of administration of the lethal agent, the temperature, and the intratracheal pressure. In the present study it was noted that the lung weight increased only slightly after death in some animals and markedly in others (Chapter 4) The rate of the postmortal increase of the lung weight is influenced by factors which are insufficiently known The position of the body after death seems to play a part as the lowermost parts of the lungs are always the heaviest postmortally Marked hyperaemia in the lungs at the moment of death may conceivably increase the tendency to postmortal pulmonary oedema (Chapter 4) Since postmortal transport of blood or serum to the lungs is probably a pre requisite for postmortal pulmonary oedema (Chapter 5) postmortal clotting time and pressure in the blood vessels could be important factors. Overhydrated rigid animals were found to have the highest lung weights, which could indicate that overhydration is another important factor

Poulsen (206) has suggested that a weight increase in the lungs may be due to other factors besides oedema fluid, e.g congestion and inflammation. This is confirmed by the present

investigations in which animals with pneumonia (the vagotomy series and 3 rabbits with pneumonia whose ureters had not been ligated) pulmonary haemorrhages (notably the dextran series) and hyperaemia (the adrena line and the vagotomy series, and some animals that died spontaneously from injection of air intravenously or 'Narkotal' injection) had lung weights exceeding the upper limit of normal range. In animals with white-spotted lungs, seen after overhydration, the lung weights were not increased

In association with experimentally produced non-focal pulmonary oedema without hyperaemia, pulmonary haemorrhages, or postmortal changes of any noteworthy degree the lung weight is a good method for evalua tion of pulmonary oedema The longer the interval after death the less the possibility to diagnose or quantitate pulmonary oedema by using the lung weight in non bled animals but the presence of pulmonary oedema can be excluded if the lung weight after death is low

The water content of the lung

Determination of the water content in the lung has been made by various techniques and opinion differs as regards the usefulness of th method (Chapter 4) The heat lamp technique does not seem to have been used for determination of the water content of the lung in experimental pulmonary oedema. This technique seems to work well for the drying of l ng tissue but is less expedient for the drying of whole blood (Chapter 4 Chapter and Table 7) It will be seen from Tables 10—25 that there was a good correlation be tween the water content and the a mount of oedema fluid noted other wise The water content alters very little at increased body weight, as has been shown in mice by Poulsen (200) The method has some disadvantages, however·

The determination of the water content uses up one lung. When gross pul monary changes involve predominant ly one lung which happens in rare cases a choice must be made between water content and histological veri fication (Reichsman [213])

It will be seen from Tables 10—25 that several overhydrated rabbits had increased water contents in their lungs, up to 85 % without showing any other evidence of pulmonary oedema. In these cases, the fluid was possibly present in blood and lung tissue and did not occur in the alveoli. In highly over hydrated animals the water content will therefore be a less reliable criterion of pulmonary oedema.

Poulsen (200) showed that in mice the water content of the lung averaged 77 11 % of blood 78.5 % and of oedema fluid from the lung 94.9 % He concluded that it seems evident that the water content of lungs showing a weight increase due exclusively to an increased accumulation of blood does not differ essentially from that of normal lungs On the other hand, a weight increase caused by oedema fluid will raise considerably the water content of the lungs. Poulsen consider ed that the water content could there fore be a useful means of diagnosing

pulmonary oedema against hyperaemia and inflammation. The present investigation has confirmed the low *water content of the lung in pneumonia* (the vagotomy series and 3 animals with pneumonia whose ureters were not tied) haemorrhages into the lung parenchyma (the dextran series) and hyperaemia (the adrenaline and the vagotomy series, and some animals that died spontaneously after injection of Narkotal or air intravenously) as well as the high water content of the *lung in cases of seemingly uncomplicated* pulmonary oedema Atelectatic lungs of animals with profuse pleural effusion following overhydration on the other hand were in many cases *rich in fluid. White spotted lungs*, which were seen in overhydrated animals, showed in many cases an increased water content (Diagram 15)

Thus, the water content is useful *in the differential diagnosis* between pulmonary oedema on one hand and hyperaemia pneumonia and haemorrhages into the lung parenchyma on the other Pulmonary oedema cannot be safely excluded on the basis of a normal water content, for in pulmonary oedema with co-existing hyperaemia haemorrhage or pneumonia the water content can be normal. In several rabbits belonging to the dextran *Narkotal* and *vagotomy* series the water content was certainly normal but they had pulmonary oedema evidenced by four or five other criteria. The alveolar oedema fluid may be fibrinous, it may have a high protein content and hence a low water content, or it can be sparse in relation to blood cells and inflammatory tissue Consequently a low water content of lungs with high weights is a guide to diagnoses other than pulmonary oedema, and was thus used in the vagotomy series as a means of excluding other pulmonary processes. For further discussion, see Chapter 9

In comparison with the postmortal increase in the lung weight, the rise in the water content post mortem was moderate (Chapter 5) In 71 % of 21 rigid, non overhydrated rabbits the *water content was below the upper* limit of normal range for spontaneously dead rabbits 38 % had a lung weight below the upper limit of normal range. In no case did the water *content post mortem exceed 86.3 %* as it may do in rabbits with moderate pulmonary oedema A high water content should therefore be more useful than a high lung weight as supporting *evidence for the presence of* pulmonary oedema in vivo in rigid animals. In some rigid animals overhydrated in vivo however the water content reached 88 % (Diagram 17) and this fact limits the usefulness of the water content in the diagnosis of pulmonary oedema in overhydrated rigid animals.

The method is particularly valuable in the differential diagnosis

Estimation and measurement of the water content The water content of the lung was estimated roughly at autopsy by inspection of the lung surface the section surface, and the amount of fluid obtained by squeezing and cutting of the lung The *estimated water content was graded as* + ++ and +++ Comparison was made between this gradation and the water content *measured by drying of the lung*. On

451 occasions the correlation was 0.62 ±0.02. A water content higher than the estimated one was found chiefly in some overhydrated animal and also in some vagotomized animals. A water content lower than that expected from th estima tion was recorded for spontaneously dead animals and animals with hyperaemia, pulmonary haemorrhages, and bronchiolitis.

Microscopical studies

According to Visscher et al. (246) microscopical studies have proved dis appointing in experimental lung oedema. They frequently sectioned lungs *with acute* massive oedema and found *that* there was no stainable material in the alveoli. Koenig (147) showed that in early acute lung oedema the oedema fluid may be nearly protein-free According to Visscher et al., the only *early evidence* of such lung oedema that is visible microscopically is inter stitial wall thickening, because protein-free fluid in alveoli is undetectable in conventional histological preparations.

In this study as well, the *microscop*ical picture was virtually normal (*even* without interstitial wall thickening) in animals which had marked pulmona ry oedema by all the other criteria (Figs. 26 and 27) In animals with marked pulmonary oedema following infusions of large doses of dextran and in overhydrated rabbits with pulmonary oedema in the adrenaline and the vagotomy series the absence of alveolar oedema was particularly striking Although there was no precipitated protein in the alveoli, the shape of the alveoli, the thickness of the alveolar septa, and the presence of red cells and alveolar phagocytes in the alveoli could in some cases give rise to the suspicion of pulmonary oedema Since however these findings are not specific for pulmonary oedema, the presence of visible precipitated protein in the al veoli (alveolar oedema) was the only factor considered here Some experi ments failed in which an attempt was made at obtaining better visualization of protein poor alveolar fluid by using a different fixative (*Carnoy's fluid*)

Moreover alveolar oedema was seen around haemorrhagic *areas* and foci of infection, and when numerous *small* foci were present as in the vagotomy Narkotal' and dextran series, the dif ferential diagnosis against alveolar oedema *of the* haemorrhagic lung oedema *type was often difficult*. In this situation it might *have been* ex pedient to give antibiotics to vagotomized *animals* as an attempt at over coming the pneumonia. Antibiotics *were given for this* purpose by Alwall et al., for instance to Narkotal' treated animals. As regards the differential di agnosis, the greatest difficulties were presented by the vagotomy series, in *which some* animals had co-existing pulmonary oedema, pneumonia ate lectasis, hyperaemia, and haemor *rhages. In this* series it was also very *difficult, to estimate microscopically* the extent of pulmonary oedema. In *spite* of these shortcomings, the microscopical examination was in many cases of decisive importance in establishing the type of pulmonary changes.

The dextran, Narkotal *adrenaline and* vagotomy series included animals which had alveolar oedema but in

pulmonary oedema against hyperaemia and inflammation. The present investigation has confirmed the low water content of the lung in pneumonia (the vagotomy series and 3 animals with pneumonia whose ureters were *not tied)* *haemorrhages into the lung* parenchyma (the dextran series) and hyperaemia (the adrenaline and the vagotomy series, and some animals that died spontaneously after injection of Narkotal or air intravenously) as well as the high water content of the lung in cases of seemingly uncomplicated pulmonary oedema. Atelectatic lungs of animals with profuse pleural effusion following overhydration on the other hand were in many cases *rich in fluid*. *White spotted lungs,* which were seen in overhydrated animals showed in many cases an increased water content (Diagram 15).

Thus the water content is useful in the *differential diagnosis* between pulmonary oedema on one hand and hyperaemia pneumonia and haemorrhages into the lung parenchyma on the other. Pulmonary oedema cannot be safely excluded on the basis of a normal water content for in pulmonary oedema with co-existing hyperaemia haemorrhage, or pneumonia the water content can be normal. In several rabbits belonging to the dextran Narkotal and vagotomy series the *water content* was certainly normal but they had pulmonary oedema evidenced by four or five other criteria. The alveolar-oedema fluid may be fibrinous, it may have a high protein *content* and hence a low water content or it can be sparse in relation to blood cells and inflammatory tissue. Consequently *a low water content of lungs* with high weights is a guide to diagnoses other than pulmonary oedema and was thus used in the vagotomy series as a means of excluding other pulmonary processes. For further discussion see Chapter 9.

In comparison with the postmortal increase in the lung weight the rise in the water content post mortem was moderate (Chapter 5). In 71 % of 21 rigid, non-overhydrated rabbits the *water content was below the upper* limit of normal range for spontaneously dead rabbits. 38 % had a lung-weight below the upper limit of normal range. In no case did the water *content post mortem exceed 88.3 %* as it may do in rabbits with moderate pulmonary oedema. A high water content should therefore be more useful than a high lung weight as supporting *evidence for the presence of pulmonary* oedema in vivo in rigid animals. In some rigid animals overhydrated in vivo however the water content reached 88 % (Diagram 17) and this fact limits the usefulness of the water content in the diagnosis of pulmonary oedema in overhydrated rigid animals.

The method is particularly valuable in the differential diagnosis.

Estimation and measurement of the water content. The water content of the lung was estimated roughly at autopsy by inspection of the lung surface, the section surface, and the amount of fluid obtained by squeezing and cutting of the lung. The estimated water content was graded as +, ++ and +++. Comparison was made between this gradation and the water content measured by drying of the lung. On

the chest was contracted (muscle cramp) and large when it was expanded.

Discussion

"The quantitation of the edema state is a difficult problem at any site. It is particularly so in the lung because the organ is relatively inaccessible in the intact animal and because standards of reference are difficult to establish (Visscher et al.) In my experience, the difficulty in establishing which animals have and which have not pulmonary oedema is due to three factors

a) There is no available method by which, under any conditions, pulmonary oedema can be confidently diagnosed (Table 26) So as not to be too dependent upon one method with its weak points, and to ensure better support for the diagnosis in doubtful cases, I have used six methods simultaneously for the diagnosis of pulmonary oedema.

b) The borderlines are vague. With the requirement that the diagnosis should be incontestable many cases of slight pulmonary oedema would be excluded and, again, if all mild cases as well were to be taken into account, some doubtful borderline cases would be included. The use of both methods with all transitions has been reported in the literature In this investigation it was required that three out of six given criteria should be positive The reasons why the number of positive criteria required was not fixed at four five, or six were the desire to consider less extensive pulmonary oedema, as acute violent forms were rare in these series, and the risk that if any one method of examination was incidentally missing or gave a false negative result, cases of frank pulmonary oedema would not have been included.

c) Many pulmonary processes can coexist with pulmonary oedema. This difficulty is most readily overcome if the nature of these processes is known. A method for diagnosis can then be chosen which gives different results for pulmonary oedema and the respective concurrent process (e g the water content in association with pulmonary oedema and with pneumonia) If many pulmonary processes of varying extent co-exist, as in vagotomized animals, the difficulties can be great. Several criteria are then necessary including microscopical evidence, to enable a reasonably confident diagnosis.

To choose the best method for the detection of pulmonary oedema, with few missed and few wrong diagnoses, is difficult. The reason for this is that the diagnosis of pulmonary oedema is difficult in the individual case, and the advantages and disadvantages of the methods do not have the same influence under different experimental conditions (Diagram 18) It will be seen from this diagram that different methods of examination give a different frequency of pulmonary oedema, that the correlation between them varies with different forms of pulmonary oedema, and that, with respect to the results, no one procedure truly agrees with the method of using three positive criteria for the diagnosis of pulmonary oedema.

which the diagnosis of pulmonary oedema was not supported by a sufficient number of other criteria. An explanation of this besides the one given above was that the changes occurred locally in one part of the lung and that specimens for microscopical examination were taken from this part.

Quantitative and qualitative evaluation of the alveolar oedema was made only from the macroscopically most severely changed part of the lung and the gradation will therefore mirror the maximal rather than the average amount of alveolar oedema. Even with the naked eye it could be seen on the slides that accumulation of oedema fluid was greater in the hilar region than peripherally towards the lung margins Specimens from both the upper lobe and the basal parts in the same rabbit showed regularly more alveolar-oedema fluid basally

The lungs of rabbits with no gross abnormalities no frothy fluid, and normal lung weight were as a rule not examined microscopically Specimens from such lungs were taken in each series, however and a total of 75 seemingly normal lungs were examined. In 44 of these the microscopical picture was also wholly normal Among the remaining 31 were seen areas of bronchiolitis in 9 small areas of atelectasis in 8 a few focal haemorrhages in 6 slight hyperaemia in 4 areas of bronchitis in 3 and interstitial periarterial oedema in 1 None of these rabbits exhibited alveolar oedema If all the unexamined lungs (125 cases, Tables 10—25) had contained alveolar oedema fluid, only 3 cases would have been diagnosed as pulmonary oedema by three criteria as required here.

In postmortal pulmonary oedema the alveolar oedema was protein rich, the alveoli rarely contained red blood cells and alveolar phagocytes, and even peripheral alveoli could be filled with oedema fluid (Chapter 5) In spontaneously dead warm and soft rabbits the presence of thin protein poor oedema fluid in a great number of alveoli in the hilar or the basal region, containing red cells and alveolar phagocytes, may support the suspicion that pulmonary oedema was present in vivo and not only post mortem.

Microscopical examination is of value in the differential diagnosis and less useful in the diagnosis and gradation of pulmonary oedema

Lung-volume and specific gravity

The method was used initially in this study but was abandoned for several reasons The air disappeared relatively quickly even from an intact lung and very quickly at the least leakage, which easily occurred. In non-bled animals the lung weight increases after death, at the same time as the air content, and hence the volume, decreases, so that the specific gravity will be greatly dependent upon the length of time that elapses between death and autopsy Atelectasis, pneumonia, and other pulmonary processes cannot be detected by this method. Increased lung volume can occur without pulmonary oedema, and pulmonary oedema can occur in a normal sized lung A lung that has been immersed in water for a while would hardly provide suitable material for determination of the water content The lung volume varied with the method of sacrifice, in that the lungs became small when

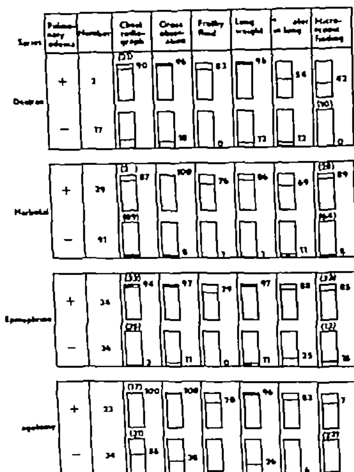


Diagram 18. Data from the dextran, "Narkotal" epinephrine, and gotomy series. The number of rabbits with and without pulmonary edema, respectively. In the three first-named series, three criteria were sufficient to establish the diagnosis of pulmonary edema, whereas in the agotomy series the three criteria of pulmonary edema should include microscopical alveolar edema or water content above the upper limit of normal range. The occurrence of the following criteria is expressed as percentage (shaded black parts of the bars). Pulmonary changes which may represent edema (chest radiograph); macroscopical changes at autopsy which indicate pulmonary edema (gross observation); *frothy fluid in the air way*; lung-weight in g per kg body weight above the upper limit of normal range (lung-weight); water content of the lung above the upper limit of normal range (% water in lung) and microscopical lveolar edema (microscopical findings). The occurrence of these criteria is also expressed numerically. The figures in parentheses denote the absolute number of nimals in each group when it deviates from the numbers set out in the column headed "Number".

Tablo 28. *Schematic survey of the sources of error in the diagnosis of pulmonary oedema in these experimental series*

	Dl g stl m th d					
	Chest X-ray	Gross bservation	Frothy fluid l the air-way	Lung weight	Water content in the lung	Microscopical findings
Non-diagnosed pulmonary oedema	Focal Basal Very acute		Small amount in the bronchi Spontaneous death before formation f frothy fluid Viscid oedema fluid? In vivo	Focal	Haemorrhagic? Hyperaemic? Focal	Protein poor oedema fluid
Other pulmonary changes wrongly diagnosed as pulmonary oedema	Atelectasis (pleural effusion) Bleedings in the lungs Hyperaemia Pneum nia	Hyperaemia Numerous small haem rrhages Coexisting mixed lung processes, bleedings, hyperaemia, pneumonia nd atelect sis (Postm rtal oedema)	Bronchitis Stagnation of secretion Bleedings in the air ways	Pneumonia Hyperaemia Bleedings in the lungs (Postmortal oedema)	Increase of extra alveolar fluid in the lungs (Postmortal oedema)	Numerous small haemorrhages or small areas of pneumonia (Postmortal oedema)

the other of pulmonary oedema with evidence of for instance large haemorrhage)

The following facts favour the three-criteria method a) The dependence upon one method with its shortcomings will be less (no one method would have been successful on all occasions in these experiments) b) Rabbits with two out of six criteria of pulmonary oedema seldom seemed, at subjective estimation, to be cases of pulmonary oedema after all" c) Rabbits with hyperaemia or haemorrhages and three positive criteria of pulmonary oedema had such marked hyperaemia or such profuse haemorrhages into the lung parenchyma that the probable borderline was attained between hyperaemia and pulmonary oedema and between haemorrhages and haemorrhagic pulmonary oedema, respectively The method failed, however with respect to pneumonia (see the vagotomy series)

Another possibility that of combining the water content and the lung weight, has been suggested by Poulsen (206) In Diagrams 14—17 are listed 745 rabbits (from preliminary experiments as well) whose lungs were examined by both lung weight and water content. To illustrate the influence of different pulmonary processes on the lung weight and water content the essential pulmonary change (diagnosed microscopically and/or macroscopically) in each rabbit has been marked out as follows A dot under the letter indicates that the rabbit had three positive criteria of pulmonary oedema but that some other

Diagram 15. The predominating types of pulmonary changes observed by macroscopical and/or microscopical examination t lung weight below 6.60 per kg body-weight and a water content above 81 % A = telectasis, B = bleedings, M = postmortal cha ges, N = normal, W = white-spotted lung surface. Of these, "W" refers to gross examination alone The presence of t least three criteria of pulmonary edema is indicated by dot under the respective letter

pulmonary change predominated It will be seen from Diagram 15 that high water contents and normal lung weights can occur in rabbits whose lungs are normal macro- and microscopically and in those that have the type of pulmonary changes characterized by atelectasis haemorrhages, and white-spotted lung surface. There is only 1 case of suspected pulmonary oedema in this group Diagram 16 shows the dominating pulmonary change at high lung weight but normal water content. This group includes cases of pneumonia haemorrhages, hyperaemia and postmortal changes but few cases of outspoken pulmonary oedema. There are, how

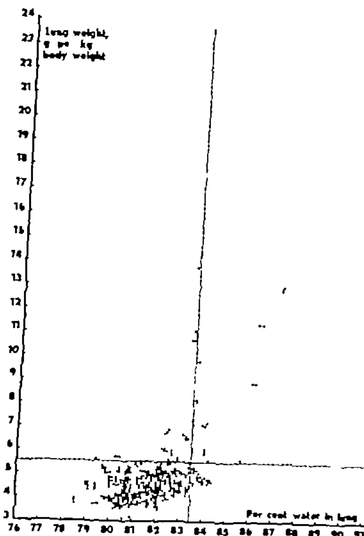


Diagram 14 Relation between lung weight in g per kg body weight and the water content of the lung as a percentage in 745 rabbits from all the experimental series

With the method of taking three criteria out of six as evidence for pulmonary oedema, the following differential-diagnostic difficulties arose *Pneumonia* (the vagotomy series) can cause an increased lung weight frothy fluid in the air ways as well as radiological macroscopical and microscopical changes which may also occur in pulmonary oedema *Haemorrhage* into the lung parenchyma (the dextran series) may increase the lung weight and give rise to macroscopical and radiological changes as in pulmonary oedema. *Hyperaemia* can cause increased lung weight slight macroscopical and presumably slight radiological changes So the problem of differentiating completely between rabbits with and rabbits without pulmonary oedema is not solved with the technique of using three positive criteria Moreover two rabbits with pulmonary oedema evidenced by three though different criteria may exhibit a completely different microscopical picture (in one it may be that of slight uncomplicated pulmonary oedema in

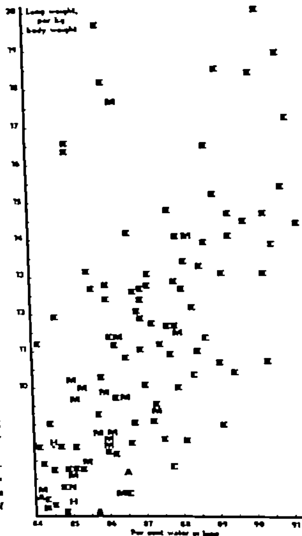


Diagram 17 Predominating types of pulmonary changes observed by macroscopical and/or microscopical examination at lung-weight over 6.5 g per kg body-weight and water content above 84 %. B = bleeding, E = edema, H = hyperemia, M = postmortal changes, N = normal. The presence of at least three criteria of pulmonary edema is indicated by dot under the letter H

have not been included in Diagram 16 and 17 respectively

Diagrams 15—17 support the view that a combination of lung weight and water content of the lungs forms a good basis for evaluation of pulmonary oedema but that in marked hyperaemia, pulmonary haemorrhages, and/or pneumonia further criteria, notably microscopical evidence, may be necessary as a means of establishing the presence of pulmonary oedema and evaluating the oedema state with reasonable probability



Diagram 16. Predominating pulmonary changes observed by macroscopical and/or microscopical examination at a l ng weight over 6 g per kg body weight and a water content below 83 5 % B=bleeding, E=edema, H=hyperemia, M=postmortal changes, N=normal, P=

ever a great number of rabbits with pneumonia hyperaemia, or haemorrhages which had three to six positive criteria of pulmonary oedema (indicated by a dot under the letter) Accordingly the water content can be normal in spite of macroscopical and radiological changes as in pulmonary oedema frothy fluid in the air ways, alveolar oedema microscopically and increased lung weight. The question as to in which of these cases pulmonary oedema actually co-existed with pneumonia hyperaemia or haemorrhages is difficult to answer conclusively Diagram 17 shows the predominant pulmonary change in rabbits with high lung weights and high water contents. Pulmonary oedema by gross and microscopical evidence was the predominant change even though hyperaemia and haemorrhages were features of the picture in most of these cases. Postmortal pulmonary oedema was mostly found in association with a low water content but the highest water contents were noted in 5 severely overhydrated (25 % of the body weight) rabbits which after a few days of uraemia died in uraemia and were examined about 12 hours after death. For want of space, 1 rabbit with pneumonia a lung weight of 18 g per kg body weight, and a water content of 81 % and 2 rabbits with pulmonary oedema and lung weights of 23 g and 24 g per kg (Diagram 14)

---

pneumonia. The presence of at least three criteria of pulmonary edema i indicated by dot under the letters B, E, H nd P

CHAPTER II

The uremia factor and the fluid factor

The uraemia factor

Under these experimental conditions, uremia per se seems not to be sufficient to cause pulmonary oedema other factors must be present.

The question whether increasing uraemia would increase the susceptibility to pulmonary oedema, was studied by experiments on rabbits in which, after bilateral ureteral ligation, lung oedema was produced by four methods known to be expedient for this purpose. It was found that increasing uraemia increased the tendency towards lung oedema after infusions of dextran solution and after Narkotal' anaesthesia whether the animals were overhydrated or not the above mentioned effect of uraemia was insignificant in non-overhydrated vagotomized rabbits and absent in the experimental series of overhydrated vagotomized rabbits, as well as after adrenaline injection, whether the animals were overhydrated or not.

The results seem to give rise to the following speculations

a) Perhaps high-degree uremia increases the susceptibility to pulmonary oedema under certain conditions only such as an enlarged plasma volume (the dextran series) membrane changes (the Narkotal series) or air way obstructions, etc. (the vagotomy series)

b) The term uraemia is used here as a synonym of the disorders that arise after ureteral ligation, for instance an elevated N P.N level, disturbances of the acid base and the electrolyte balance the uraemia damages mucous membranes and the myocardium and causes respiratory and circulatory disturbances. It is not possible to single out any one of these factors as being especially capable of predisposing the animals to pulmonary oedema. On the other hand, ascites occurs after ureteral ligation the tendency towards formation of ascites results in deplacement of fluid to the peritoneal cavity at overhydration, whereby the risk of pulmonary oedema may be reduced.

c) Unspecific factors may influence the frequency of pulmonary oedema under otherwise similar conditions. The fact that highly uraemic animals were more markedly affected by the Narkotal' narcosis than slightly uraemic ones may possibly have contributed to the higher frequency of pulmonary oedema in the first mentioned

Range of usefulness of different methods for examination

From the experience gained in these experiments the methods of examination applied here seem to have the following range of usefulness

1 The method for the diagnosis of pulmonary oedema must be chosen with a view to the purpose of the investigation and the method used in producing pulmonary oedema.
2 At in vivo investigations, X ray examination should be used and checking made by "clinical" assessment
3 In acute violent pulmonary oedema evaluation of frothy fluid in the air ways seems to suffice
4 When the recording of insignificant pulmonary changes is also desirable and/or great exactness is not required simple assessment of the gross appearance of the lungs and the presence of frothy fluid is preferable
5 If during the experiments, pulmonary oedema and pneumonia or haemorrhages in the lungs should occur only to a small extent and the animals can be bled lung weight measurement is the superior method for evaluation of pulmonary oedema
6 If the experimental conditions are such that not only pulmonary oedema but also hyperaemia pulmonary haemorrhage, and/or pneumonia may be thought to occur determination of the water content of the lungs is the best method in combination with lung weight measurement and, if desired, microscopical examination.
7 If several different pulmonary processes in various combinations should occur as in vagotomized animals microscopical examination is necessary
8 In rabbits with rigor mortis, pulmonary oedema developed in vivo can be suspected when there are profuse frothy fluid in the air ways, distinctly increased water content in the lungs, and thin alveolar-oedema fluid with alveolar phagocytes and red cells in the alveoli and when there are bilateral changes consistent with pulmonary oedema localized mainly in the hilar region.
9 Pulmonary oedema in normal or slightly ill rabbits can most readily be excluded by clinical assessment
10 In increasing uraemia a slight rise in the frequency of haemorrhages, atelectasis, and pneumonia was noted in some series. Therefore the need of several methods including microscopical examination and estimation of water content increased with increasing uraemia in these series.
11 Only by using several methods simultaneously can a fairly exact *qualitative and quantitative* evaluation of pulmonary oedema be made in rabbits

came very poor after amounts equal ling only 15 % of their body weights. This can be due to the effect of such factors as haemolysis, cerebral oedema, intoxication, hyperglycaemia or hyponatraemia. Pulmonary oedema was not noted.

The experimental conditions limit to some extent the possibilities to draw a conclusion about uraemia as a predisposing factor to pulmonary oedema. In some experiments the overhydration was carried out such a long time after the ureteral ligation that the remaining length of life might have been too short for pulmonary oedema to develop.

The conclusion that can be drawn from these experiments is, mainly that overhydration in association with uraemia in animals with both their ureters ligated produced pulmonary oedema only if the overhydration meant an increase of the blood volume. Other factors than uraemia must be present. Salt and fluid retention increased the susceptibility to pulmonary oedema after Narkotal narcosis, adrenaline injection, and vagotomy in uraemic rabbits.

group The stress of the adrenaline effect in already severely ill animals may shorten their lives so that there will be no time for pulmonary oedema to develop before death occurs The same may be the case after vagotomy particularly in those groups in which the operation was made a relatively long time after the ureteral ligation that is, in cases of severe uraemia and/or serious infectious complications which possibly could have been prevented by antibiotic treatment.

These considerations lead to the conclusion that uraemia following ligation of the ureters can be a factor which under some conditions increases the frequency of pulmonary oedemea and under other conditions lessens it

The fluid factor

After vagotomy Narkotal narcosis and adrenaline injection animals with salt and fluid retention developed pulmonary oedema more readily than did animals without salt and fluid retention irrespective of the degree of uraemia This confirms other authors observations that salt and fluid retention increases the susceptibility to pulmonary oedema under various experimental conditions In the 3 experimental series mentioned above the degree of overhydration was relatively low the amount of administered fluid was 15 % of the animals body weight (less than 1 7 ml per kg body weight were infused per minute)

Despite administration of blood in amounts that were twice those of the animals circulating blood, slight pulmonary oedema developed in only a few cases On the other hand, when the plasma volume was increased by the infusion of dextran the frequency of pulmonary oedema produced was higher than after administration of the same amount of blood. This may be interpreted as showing that an increase of the plasma volume is the essential factor in the development of pulmonary oedema at overhydration. The result may however depend upon other effects of the added dextran, such as anaemia due to dilution, altered capillary permeability and/or altered composition of the extracellular fluid.

The extracellular fluid was increased by infusion of mannitol solution or Ringer s solution Mannitol infusion led to hyponatraemia and had a toxic effect at dose levels of 15 % of the body weight signs of slight pulmonary oedema were noted in 1 animal in the series Ringer s solution was well tolerated and highly uraemic animals even improved after infusion of amounts equalling 15 % of their body weights pulmonary oedema did not develop Nor did pulmonary oedema develop even if the amount of Ringer s solution was increased to 50—100 % of the body weight in moderately uraemic animals, provided that the infusion was not given too rapidly Alwall & Lindqvist also found that high salt and fluid retention only in rare cases caused slight pulmonary oedema in uraemic rabbits

When the total amount of fluid was increased by infusion of urea or glucose solution, the animals died or be-

to establish the diagnosis of pulmonary oedema. Chest X ray gross observation, frothy fluid in the air ways, lung weight, water content of the lung and microscopical observation. In the vagotomy series supporting evidence by either microscopical findings or water content was required for the diagnosis of pulmonary oedema.

Chapter 5 The control series

Alwall et al. (16) showed that rabbits whose ureters had been tied bilaterally lived for 1—6 days after the ureteral ligation without exhibiting any radiographic pulmonary changes in vivo and that their lung weights were microscopically normal post mortem. In the present studies (78 rabbits which after ureteral ligation had anuria for 0 1—4 days) it was noted in vivo that classical signs of pulmonary oedema (dyspnoea, rattling respiration, etc.) were not present, that the respiratory rate fell with increasing uraemia, that the heart rate remained unchanged, that the frequency of E.C.G abnormalities, notably negative T waves, rose with increasing uraemia, that slight cardiac enlargement occurred in about 15 % of severely uraemic animals and that slight radiographic pulmonary changes appeared within 3 days in 3 % of the animals

Postmortally in animals killed by bloodletting, it was found that in most cases, even in severe uraemia, the macroscopical picture was normal, though in about 20 % there were a few to numerous punctiform haemorrhages and in about 3 % atelectatic areas, that the amount of ascitic fluid increased markedly with increasing uraemia (averaging after 3—4 days 108 ml) whereas the amount of pleural effusion increased insignificantly and that frothy fluid in the air ways did not occur in uraemic animals. Measurements of the lung weight in 58 bled rabbits (weighing 1.38—5.87 kg) showed that the lung weight per kg body weight falls appreciably with increasing body weight. The normal value for the lung weight of rabbits is, according to Durlacher et al (73) 3.57 ± 0.21 g per kg. Luisada (154) quotes the figure 4.55 ± 0.21 g per kg and Alwall et al (21) 4.0 (3.2—5 0) g per kg body weight (the rabbits weighed 1 72—2 40 kg). In the present experiments the lung weights in 22 bled normal rabbits weighing 2.20—3 49 kg were 4 17 ± 0.53 g per kg and in 78 bled uraemic (anuric for 0—4 days) animals 4.25 ± 0 46 g per kg with no significant increase for the most severely uraemic animals. Alwall et al. (21) in 31 animals (weighing 1.83—2.50 kg) with anuria for 2 days, found an average lung weight of 4 11 (3 4—5 7) g per kg body weight. The water content in the lungs of 59 bled normal rabbits (weighing 1.38—5.87 kg) fell on an average insignificantly with increasing body weight. The normal value for rabbits is, according to Reichman (218) 77 1 % according to Mendenhall et al. (180) 79.8 % and according to Manery et al. (176) 80.2 % the latter two percentages are means, and the authors used freeze drying and drying in an oven, respectively. In the present experiments, dry

A concise survey of literature and own experimental results

Chapter 2 Literary survey on the problems of uraemic pulmonary oedema

The clinical, radiographic, and microscopical pictures of uraemic lung oedema are described Special emphasis is placed on the difficulties attaching the differential diagnosis. The pathogenesis of uraemic pulmonary oedema *is discussed The role of fluid* retention has been established (Alwall *et al*) *but its relation to many other* possible factors such as uraemia salt retention the localisation of the fluid in the body cardiac insufficiency hypertension hypoxia anaemia cerebral factors, and hypoproteinaemia has not been made clear

Chapter 3 Literary survey on experimental uraemic pulmonary oedema

Experimental pulmonary oedema in animals can be induced in several ways. The susceptibility to lung oedema varies from species to species. Experimental studies on uraemic pulmonary oedema fluid lung and "fluid retention lung" are few only Alwall Lunderquist *et al*, and *Borgström et al* have published investigations into this subject

Chapter 4 Technique

After preliminary trials, bloodletting was chosen as the method of sacrifice, *for animals that did not die spontaneously The experiments were made on* rabbits and the following ten methods, the first four referring to in vivo observations the rest to postmortem findings were used in estimating the degree of pulmonary oedema 1) Observations on the appearance and behaviour of the animals, 2) respiratory rate measurement, *3) electrocardiography* (heart rate and E C G disturbances) 4) chest X ray 5) gross observation 6) observation and collection of frothy fluid in the air ways postmortally 7) measurement of lung weight absolutely and in relation to body weight, *8)* estimation of water *content in the lung 9) microscopical* observation, and 10) measurement of lung volume in a few experiments. The literature dealing with the technique used at these procedures is surveyed. In the present experiments a positive result *of at least three of the following examinations was required*

changes were slight. As regards the respective criteria of pulmonary oedema, it may be noted that, for instance, gross haemorrhages in the lungs were seen in 62 % that a high water content was rare (1 case) with this form of overhydration, that chest radiography showed slight pulmonary changes, possibly ascribable to hyper aemia, in 33 % immediately after the transfusion and in 14 % immediately before the bloodletting, and that mi croscopical examination showed peri arterial interstitial oedema in 2 cases and relatively large numbers of leucocytes in 4 cases.

Increase of blood volume by dextran infusion

Intravenous infusion of 2 ml of dex tran solution per minute and kg body weight for about 2 hours did not produce pulmonary oedema in dogs (258) In the present study 6 % dextran solu tion 12 % of the body weight, was infused for 1 /s hours in 41 rabbits whose ureters had been tied bilaterally Animals that did not die spontaneously were bled after 4—5 hours. Pul monary oedema evidenced by three criteria developed in 2 out of 11 rabbits with anuria for less than 6 hours, in 6 out of 10 with anuria for 24 hours, in 7 out of 10 with anuria for 48 hours, and in 9 out of 10 with anuria for 72 hours. The frequency of pulmonary oedema increased significantly with increasing uraemia. After infusion of dextran in uraemic rabbits the frequency of pulmonary oedema was hig ber than after transfusion of the same amount of blood

The six criteria of pulmonary oede ma used here agreed less well in this series than in the others the diagnosis of pulmonary oedema was made in 24 cases and was supported by all the six criteria in only 8 % and by five cri teria in 43 % The low percentages are due mainly to the failure of demon strating alveolar oedema in 57 % of the 21 cases with at least four criteria of pulmonary oedema, and secondly to the fact that the water content was normal in 46 % of the cases with at least three criteria of pulmonary oedema. The great diagnostic difficulty in this series was to distinguish between pulmonary oedema and pulmonary haemorrhage, as haemorrhages of varying degrees were recorded in 98 % and less extent between pulmonary oedema and hyperaemia It was therefore particularly difficult to establish the presence of pulmonary oedema on the basis of the gross picture and lung weight, but also radiologi cally

Increase of the extracellular fluid by infusion of saline

The production of pulmonary oedema in rabbits by infusion of saline intra venously requires an infusion rate of at least 3 ml per minute and kg (53) Pulmonary oedema can be produced with a lower infusion rate if some other factor co-exists which in itself does not have a sufficient oedema producing effect. (17 49 52 149 150) Fluid retention alone has not been found to produce pulmonary oedema in animals. Alwall et al. (17) infused

ing was done with a heat lamp and the water content was found to be 81 0±0 0 % in normal animals and *80±1.3 % in 78* uraemic (anuria for *0—4 days) animals* Microscopic exa *mination showed* no abnormalities in *12 rabbits with* anuria *for 3—4 days focal haemorrhages* in 3 one of which had atelectasis as well

Darlacher et al (73) found that congestion and oedema develop in the postmortem period in rabbits. I have examined *30 rabbits which died* spontaneously in uraemia the examination was carried out before rigor mortis had set in, *5—90 minutes after death* Frothy fluid was not found the lung weight averaged 4 08±0 64 g per kg *and the water content* 80 9±1 5 % (these figures were used as control values for spontaneously dead ani*mals) Slight postmortal changes* with hyperaemia and slight alveolar oedema were seen in 3 of the rabbits

The postmortal changes were still more marked in animals that were examined 24 hours after death In this group the lung weight was 7 71±2 80 g per kg body weight and the water *content 82 6±2 5 % (21 rabbits which* died spontaneouly in uraemia) Frothy fluid in air ways was noted in 19 % *(52 rabbits including 31 killed by in*jection of air) Gross observation showed either bright red light lungs or heavy dark red lungs in some cases with mottled symmetrical markings towards the periphery which made it impossible to establish whether pulmonary oedema had developed in vivo *Microscopically there were profuse to* moderate protein rich type alveolar oedema and hyperaemia but red cells *and alveolar phagocytes were rarely seen in the alveoli.*

There was no significant difference in the lung weight *or the water* content between 16 normal and 15 severely uraemic rabbits 24 hours after they had been killed by *intravenous injec*tion of air

Thus in these experiments no tendency towards *pulmonary* oedema could be demonstrated, neither in rabbits with increasing uraemia after ureteral ligation nor in rabbits that died in uraemia Uraemic rabbits have no *significant tendency to develop* post mortal pulmonary oedema more frequently than normal rabbits.

Chapter 6 Pulmonary oedema and overhydration

Increase of the blood volume by blood transfusion

In dogs a blood transfusion amounting *to 17 %* of the body weight for ? hours does not necessarily produce lung oedema (129) In the present ex*periments, blood transfusions* 12 % of the body weights were given for 1 $^{1}/_{2}$ hours intravenously to 21 rabbits whose ureters had been tied bilaterally including 11 which had been anuric for a few hours and 10 with anuria *for* 48 hours No reactions were noted in vivo The animals were bled 4 $^{1}/_{2}$ hours later At least three criteria *of* pulmonary oedema were satisfied in 1 out of 11 rabbits with anuria *for* a few hours and in 2 out of 10 which *had* been anuric *for* 48 hours the pulmonary

Thus, the toxicity of the mannitol solution rendered the assessment of its oedema producing effect difficult, but this effect seemed to be slight.

Increase of all the fluid spaces by infusion of glucose solution

Administration of 150—300 ml of 5 % glucose solution per kg and hour did not produce pulmonary oedema in dogs (257) Administration of large amounts of water through a gastric tube to rabbits produces fatal cerebral oedema (93) The present author infused 5.5 % glucose solution intravenously 15 % of the body weight, for 1 1/ hours in 28 rabbits whose ureters had been tied. As the blood sugar level became normal, signs of water intoxication appeared. Four rabbits died during the infusion, 9 were bled within 5—7 hours and 10 within 24 hours of the infusion. Five rabbits died spontaneously within 10—23 hours of the infusion. One rabbit which died during the infusion had slight pulmonary oedema the others did not have three criteria of pulmonary oedema.

Increase of all the fluid spaces by infusion of urea solution

Daily administration of 200 ml of 10—20 % urea solution intravenously to rabbits whose ureters had not been tied led to high concentration of urea and death of the animals after some time hypertension was noted in vivo and post mortem the lungs showed hyperaemia but no pulmonary oedema (236) Aqueous solutions of urea produce haemolysis, both in concentrated and in diluted solutions, whereas 25 % urea solution in, for instance isotonic glucose does not (218) The present experiments with infusion of urea solution comprise 4? rabbits with tied ureters. Isotonic (1.8 %) urea solution infused intravenously in amounts equalling 10—15 % of the body weight was toxic to uraemic rabbits which had been anuric for 48 hours, whether the solvent was water 5.5 % glucose, or 10 % invertose, and even when the infusion was given slowly by the subcutaneous route. Slightly uraemic rabbits, on the other hand, tolerated slow subcutaneous infusion of this amount of isotonic urea solution. Anuric rabbits died at an N.P.N. level of 300—400 mg per 100 ml, even when they were not over hydrated and the rise in N.P.N. was largely attributable to urea.

Pulmonary oedema as defined here occurred in 2 animals which were given 50 % urea solution by rapid intravenous infusion but did not occur in animals which were given isotonic urea solution or 30 % urea solution for 30 minutes.

Chapter 7 Pulmonary oedema following anaesthesia induced with barbiturates

A technique similar to that described by Alwall (13 16) was used here.

Alwall used slow injection (20 minutes) of 1.2 ml f 'Narkotal' solution per kg body weight. In the present experiments the dose was increased to 1.7 ml per kg body weight and deep narcosis was induced for

Ringer's solution, 12 % of the body weight in rabbits whose ureters had been ligated the animals survived 6 days after the infusion. *Slight radiographic* pulmonary changes occurred in 24 % of the cases but there was no clear cut case of pulmonary oedema. In 30 animals bled after 2 days of anuria the lung *weight averaged 4 63* (3.2—6.39) kg (body weight 1 75—2.53 kg) With an increase of the dose to 25 % (Alwall et al [29]) the radiographic pulmonary changes increased to 60 % within 48 hours but no case of frank pulmonary oedema occurred *and the series was complicated by* a high frequency of profuse pleural effusion and atelectasis

The present author has continued Alwall's et al experiments by giving *infusions at varying times after the* ureteral ligation 65 rabbits in groups of 10—11 received infusions of 15 % Ringer's solution for 1 1/2 hours either 1/2 hour 24 48 or 72 hours after the *ureteral ligation* and were bled within 2—4 hours of the infusion or 48 or 72 hours after the ureteral ligation and were bled after 24 hours One rabbit *developed slight pulmonary oedema* evidenced by three criteria the animal was moribund and had severe E.C.G *changes before the infusion.* The water content of the lung exceeded the upper limit of normal range in 13 % of the animals and slight radiographic pulmonary changes were seen in 6 % No *significant* tendency to accumulation of fluid in the lungs could be noted in 62 *bled uraemic rabbits the lung* weight averaged 4.39 (3 16—5 62) g per kg and the water content *82 2* (79 2—85.2) % The administered fluid was partly *deposited as ascites* and pleural effusion Improvement of general condition fewer E.C.G abnormalities and prolongation of the survival time were noted in severely uraemic rabbits after the infusion.

Massive slow infusion of Ringer's *solution, 50—100 %* of the body weight, produced atelectasis and no pulmonary oedema in 8 highly uraemic rabbits with tied ureters which survived the infusion.

Increase of the extracellular fluid by infusion of mannitol solution

Infusion of a solution of 5 % mannitol plus 5 % sorbitol in amounts equalling 10 % of the body weight did not produce pulmonary oedema in dogs (178) *The present author infused isotonic* (5.5 %) mannitol solution intravenously 15 % of the body weight for 5 hours in 20 rabbits with tied ureters and anuria for 48 hours (the planned infusion time of 1 1/2 hours had to be abandoned, as the animals died showing *all the signs of cerebral oedema*) After the infusion the animals were weak and their respiration slow E.C.G disturbances occurred in 63 % and cardiac enlargement in 47 % The subcutis was rich in fluid and pleural effusion profuse (averaging 23 ml in bled animals) Only 3 rabbits survived 24 hours after the infusion. Pulmonary oedema evidenced by three *criteria* was present in 1 out of 10 bled rabbits but absent in 5 non rigid spontaneously dead rabbits. Pulmonary oedema of slight degrees could not be ex*cluded in 5 rigid rabbits*

Thus, the toxicity of the mannitol solution rendered the assessment of its oedema producing effect difficult, but this effect seemed to be slight.

Increase of all the fluid spaces by infusion of glucose solution

Administration of 150—300 ml of 5 % glucose solution per kg and hour did not produce pulmonary oedema in dogs (257) Administration of large amounts of water through a gastric tube to rabbits produces fatal cerebral oedema (93) The present author infused 5.5 % glucose solution intrave nously 15 % of the body weight, for 1 ¹/₂ hours in 28 rabbits whose ureters had been tied. As the blood sugar level became normal, signs of water intoxi cation appeared. Four rabbits died during the infusion, 9 were bled within 5—7 hours and 10 within 24 hours of the infusion. Five rabbits died spon taneously within 10—23 hours of the infusion. One rabbit which died du ring the infusion had slight pulmonary oedema the others did not have three criteria of pulmonary oedema.

Increase of all the fluid spaces by infusion of urea solution

Daily administration of 200 ml of 10—20 % urea solution intravenously to rabbits whose ureters had not been tied led to high concentration of urea and death of the animals after some time hypertension was noted in vivo, and post mortem the lungs showed hyperaemia but no pulmonary oedema (236) Aqueous solutions of urea produce haemolysis, both in concentrated and in diluted solutions, whereas 25 % urea solution in, for instance, isotonic glucose does not (218) The present experiments with infusion of urea solution comprise 42 rabbits with tied ureters. Isotonic (1.8 %) urea solution infused intravenously in amounts equalling 10—15 % of the body weight was toxic to uraemic rabbits which had been anuric for 48 hours, whether the solvent was water 5.5 % glucose, or 10 % invertose, and even when the infusion was given slowly by the subcutaneous route. Slightly uraemic rabbits on the other hand, tolerated slow subcutaneous infusion of this amount of isotonic urea solution. Anuric rabbits died at an N.P.N. level of 300—400 mg per 100 ml, even when they were not over hydrated and the rise in N.P.N. was largely attributable to urea

Pulmonary oedema as defined here occurred in 2 animals which were giv en 30 % urea solution by rapid intra venous infusion but did not occur in animals which were given isotonic urea solution or 30 % urea solution for 30 minutes.

Chapter 7 Pulmonary oedema following anaesthesia induced with barbiturates

A technique similar to that described by Alwall (15,16) was used here.

Alwall used slow injection (30 minutes f 1.2 ml of 'Narkotal' sol tion per kg body weight. I the present experiments the dose was increased t 1.7 ml per kg body weight and deep narcosis was induced for

the purpose of obtaining more severe changes more quickly Alwall *et al.* performed ureteral ligation under Narkotal narcosis. I tied the rabbits ureters under local anaesthesia and did not give Narkotal narcosis until 2 24 48, or 72 hours *after the ureteral ligation.* Alwall et al. bled their animals after 48 hours or let them live until they died spontaneously after 1—5 days. I bled the animals that survived 24 hours after the termination of the experiment. Alwall et al found radiographic pulmonary changes in 8 % of non *overhydrated animals which* had been subjected to ureteral ligation under Narkotal narcosis and allowed to live until they died spontaneously I found such changes in 7 % using a different narcosis technique *and bloodletting after 24 hours in* animals whose ureters had been tied. Alwall et al., in rabbits whose ureters had been tied under Narkotal narcosis and which were given an amount of Ringer s solution equalling 15 % of the body weight for 1 ½ hours noted radiographic pulmonary changes in one-third of the cases within 24 hours and in two-thirds during the observation period (1—5 days) In my experiments, using a different narcosis technique and bloodletting after 24 hours, I found radiographic pulmonary changes *in one-third of the animals which had* been subjected to ureteral ligation and overhydrated with Ringer s solution in *amounts equalling 15 % of the body* weight for 1 ½ hours. In my series the lung weights were much higher Alwall (13 17) Alwall & Lunderquist (16) and Lunderquist (171) found that the radiographic pulmonary changes *disappeared* when the administered fluid was removed by ultrafiltration and concluded that fluid retention is a basic pathogenetic factor in pulmonary changes of the type discussed here (10) The present experiments were designed to study the importance of the uraemia factor in the frequency of pulmonary oedema *following Narkotal narcosis* in non-overhydrated and overhydrated animals with tied ureters.

In these experiments the ureteral ligation was performed under local anaesthesia *(Xylocain)* and narcosis was induced with Narkotal (1 7 ml per kg body weight) 2 24 48 and 72 hours after ureteral ligation. At least half the animals were given infusions of Ringer s solution (15 % of the body weight for 1 ½ hours) immediately after completed injection of Narkotal Those that lived 24 hours after the end of the experiment were bled. Three criteria of pulmonary oedema were not satisfied in any of the 10 rabbits that had received Narkotal narcosis after *2 hours of anuria nor* in any of the 10 that were given the narcosis after 24 hours of anuria three criteria of pulmonary oedema were fulfilled in 2 of 10 rabbits given the narcosis after 48 hours of anuria and in 2 of 12 that received the narcosis after 72 hours of anuria

Among the overhydrated animals at least three criteria of pulmonary oedema were satisfied in 1 of 22 with narcosis *after 2 hours of anuria in 3* of 10 with anuria for 24 hours in 11 of 23 with anuria for 48 hours and in 10 of 22 with anuria for 72 hours *Pulmonary* oedema developed in a greater number of cases among severely uraemic rabbits (anuria for at least 48 hours) than among slightly uraemic rabbits (anuria for less than 12 hours) In the *series of overhydrated rabbits* this difference was significant. Pulmonary oedema following Narkotal narcosis developed in a greater number of cases among rabbits with salt and fluid retention than among rabbits with tied ureters without salt and

fluid retention in all stages of uraemia but the difference between the two groups was greatest in severely uraemic rabbits (p=0 00)

Bronchiolitis occurred in half the rabbits after 'Narkotal narcosis. The duration and the depth of narcosis do not seem to have influenced the frequency of pulmonary oedema. The highly uraemic animals were, however more markedly affected by the narcosis than were slightly uraemic animals, which may have been of significance as regards the frequency of pulmonary oedema. In this series, lung oedema occurred in mild degrees *here and there in the hilar or basal* regions and in such cases the diagnosis rested mainly on the gross and the microscopical findings.

Chapter 8 Pulmonary oedema following injection of adrenaline

A technique similar to that described by Drenkhahn (69) was used in producing pulmonary oedema in rabbits.

The adrenaline dose used by Drenkhahn, 3 mg per kg body weight, was in this series reduced to 1 mg per kg in view of the other experimental conditions. The latter dose caused within 20 minutes pulmonary oedema as defined here in 40 % of a control series of 10 animals. The rest of the experiments were made on rabbits whose ureters had been tied. The experiments reported in the literature do not seem to have been carried out under conditions that were similar to those used here.

Adrenaline was injected intramuscularly in non-overhydrated and overhydrated rabbits whose ureters had been tied 1—4 48, and 72 hours, respectively before the injection. Each group comprised 10 rabbits. Among these animals, which were thus slightly to severely uraemic, pulmonary oedema developed in the non-overhydrated series in 50 % 40 % and 30 % of the respective group In the control series the corresponding figure was 60 % Among animals which were overhydrated by infusion of Ringer's solution in amounts equalling 15 % of their body weights, pulmonary oedema *evidenced by at least three criteria* was noted in 60 % 50 % and 50 % respectively Accordingly the frequency of adrenaline-induced pulmonary oedema did not increase with increasing uraemia under these experimental conditions.

However in the adrenaline series the number of rabbits that died without having pulmonary oedema was higher among severely uraemic (anuria for at least 72 hours) than among the less severely uraemic (anuria for less than 72 hours) animals, or 55 % as against 16 % The explanation of this may be that in highly uraemic animals death was more often due to some adrenaline effect other than pulmonary oedema. In interpreting the results of the adrenaline series it was therefore difficult to determine whether severe uraemia leads to increased tendency towards pulmonary oedema.

The frequency of pulmonary oedema was slightly higher among overhydrated than among non-overhydrat

ed animals but the difference is not *statistically significant. It was difficult* to render the two groups comparable, for several reasons The concentration and amount of adrenaline may have been different between the groups, in that the overhydrated animals had a larger fluid space (> 15 %) and were given a higher dose (15 %) of adrenaline than the non-overhydrated animals and the rate of absorption for the intramuscular adrenaline could *have differed between the two groups*

This series illustrates the difficulties of obtaining comparable groups for the purpose of throwing light on the problems studied by these experiments

Chapter 9 Pulmonary oedema following vagotomy

The variegated microscopical picture of pulmonary changes following vagotomy *has been described by earlier* authors (168 213 246) Kraus (150) *found that infusion of saline after* vagotomy produces pulmonary oedema in rabbits Brunn s *(42) brief* report that nephrectomy prevents the development of pulmonary oedema after subsequent vagotomy and over hydration was not verified by the experiments on rabbits with tied ureters described below Experiments in animals with tied ureters do not seem to have been reported in the literature No previous author has given the whole infusion before vagotomy The risk of asphyxia in handling the animals after vagotomy must be considered.

61 rabbits were subjected to bilateral ureteral ligation, but the series was reduced to 57 since 4 rabbits were found dead after rigor mortis had already *set in and* were therefore excluded The animals were vagotomized 0—48 hours after the ureteral ligation. Immediately before the vagotomy half the animals were given infusions of Ringer s *solution in amounts* equalling 15 % of their body weights *for 1 1/2 hours Among 20 non-over* hydrated animals 15 were submitted to vagotomy within 0—4 hours of ureteral ligation and 14 within 48 hours of ureteral ligation. In 28 overhydrated animals vagotomy was performed in 14 within 3—5 hours and in 14 within 48 hours of the ureteral ligation. Those that did not die spontaneously were killed by bloodletting within about 14 1/2 hours of the vagotomy 4 moribund animals were bled within only 5—10 hours

The pulmonary changes *following* vagotomy consisted of mixed oedema *pneumonia atelectasis, hyperaemia* and haemorrhages in various combinations The method for *establishing* pulmonary oedema used in the other series namely on the basis of at *least* three criteria proved unsatisfactory in the vagotomy series. Seven rabbits with outspoken pneumonia came to be included in the pulmonary-oedema group The requirements for the diagnosis of pulmonary oedema therefore changed so that the three criteria to be satisfied were to include microscopical evidence of alveolar oedema or a water content exceeding the upper limit of normal range. Arguments in favour of this method of assessment are that

extensive pneumonia is readily diagnosed microscopically and that the water content can be presumed to be normal in cases of pneumonia (see Poulsen [211] and Diagram 17)

23 rabbits had pulmonary oedema according to this definition. In 15 of them the diagnosis was supported by at least five criteria. Among 34 rabbits without pulmonary according to this diagnosis, 80 % (27 rabbits) had at most two positive criteria, but in the intermediate group (26 % of all the rabbits) the diagnosis was difficult. In this series great uncertainty attached to all the criteria of pulmonary oedema used here.

Pulmonary oedema according to this definition was in the non-overhydrated series diagnosed in 2 out of 15 slightly uraemic animals and in 5 out of 14 severely uraemic animals in the overhydrated series it was established in 9 out of 14 slightly uraemic and 7 out of 14 severely uraemic animals. Thus, the frequency of pulmonary oedema was higher in cases of severe than in cases of slight uraemia among non-overhydrated animals and the reverse in overhydrated animals. The difference in this respect between slightly uraemic and highly uraemic animals is not statistically significant in either series. The frequency of pulmonary oedema was higher among overhydrated than among non-overhydrated rabbits, notably in cases of slight uraemia for which the difference is almost significant ($0.01 < p < 0.02$)

Another difficulty was that vagotomy lung oedema takes some time to develop Five animals died in asphyxia so soon after the vagotomy that there was reason to suspect that pulmonary oedema had not had time to develop

The problems that arose in this series may be of special interest, since a parallel can be drawn to clinical conditions where the differential diagnosis against pulmonary oedema presents difficulties in the presence of pneumonia, haemorrhages, and atelectasis (see Alwall [20 21])

Summary

In a survey of 903 patients with acute and chronic renal failure, Alwall (21) showed that the frequency of the uraemic pulmonary oedema is high (42 7 %) and emphasized that these pulmonary changes are of great importance in the diagnosis and treatment of cases in a renal medical clinic. The difficulties of making a correct diagnosis in vivo of uraemic pulmonary oedema were also discussed

I have tried to elucidate the diagnostic and pathogenetic problems of uraemic pulmonary oedema by experimental studies in animals

Detection and quantitative study of pulmonary oedema

In this study six methods were used for the evaluation of pulmonary oedema Chest X ray gross observation, observation and collection of frothy fluid in the air ways measurements of lung weight analysis for percentage water content in the lungs, and microscopical observation, and the results of these methods were compared. If it was supported by at least three of these criteria the diagnosis of pulmonary oedema was established. In the vagotomy series, the three criteria to be satisfied had to include microscopical lung oedema or a raised water content. The appearance and behaviour of the animals respiratory rate, E.C.G and heart rate, and in some experiments the lung volume were also recorded. After ligation of the ureters in uraemic rabbits pulmonary oedema was induced by hypervolaemia (blood, dextran) vasoactive drugs (adrenaline) pharmacological agents (barbiturates) and alteration of the peripheral nervous and respiratory systems (vagotomy) About half the animals were overhydrated with Ringer s solution the degree of uraemia varied from insignificant to severe. All these methods were used because the writer wished to study the importance of the uraemia factor and the salt fluid retention factor and to evaluate different criteria of pulmonary oedema under varying experimental conditions In only a few experiments was sudden pulmonary oedema induced. The object was in most cases to induce slowly supervening pulmonary oedema

The appearance and behaviour of the rabbits The observation of the animals appearance and behaviour was valuable, particularly in cases of acute

sudden pulmonary oedema, but the assessment became increasingly haz ardous the weaker the animals power of reaction and the greater the number of complications that occurred.

Respiratory rate The respiratory rate rose in association with e.g. restless ness, lung oedema, and atelectasis, and fell at rest, with increasing uraemia, after vagotomy narcosis, and premor tally as well as in the supine position. Normal restless animals could have an equally high respiratory rate as ani mals with pulmonary oedema on an average, the rate was higher in those with lung oedema, even the severely uraemic ones.

E.C.G and heart rate Most rabbits with E.C.G. disturbances had no pul monary oedema and many rabbits with pulmonary oedema had only slight E.C.G changes The heart rate also proved to be of little diagnostic value, as it changed insignificantly in association with pulmonary oedema and uraemia

Chest X ray X ray density changes could be detected even in cases of slight other criteria of pulmonary oedema. *In most cases the* X ray evid ence was well consistent with th other criteria of pulmonary oedema. The situations in which the X ray did not agree with other criteria were acute sudden pulmonary oedema (rapid in jection of barbiturates intravenously and drenaline intramuscularly) pul monary oedema with basal involve ment or in the form of numerous small circumscribed lesions (the Narkotal and the dextran series) in which very little was visible on the film, pneu monia (the vagotomy series) haemor rhages into the lung parenchyma (the dextran and the Narkotal series) at electasis (the vagotomy series, over hydrated animals) and possibly hyper aemia (overhydrated animals) in which differential diagnosis was diffi cult. Technical factors were a minor problem. Chest radiography is useful in experiments in vivo, as in Alwall's and Lunderquist s studies (15, 16, 17 21 38 171 a)

Gross observation. It was the most sen sitive of all the methods for diagnosis of experimental pulmonary oedema but also subjected to great uncertainty as regards slight pulmonary changes With the macroscopical evaluation used in this study pulmonary oedema evidenced by this criterin was diagnos ed in excess. Gross observation is use ful as a quick method which is parti cularly expedient in detecting all forms of local pulmonary changes Its in-adequacy relates to the subjective gradation and the differential-diag nostic difficulties that arise in cases of co-existent severe hyperaemia hae-morrhages into the lung parenchyma pneumonia, atelectasis, and postmortal oedema.

Frothy fluid in the air-ways The method was found to be a simple and quick aid to the diagnosis of marked pulmonary oedema. Many animals with slight pulmonary oedema or in poor general condition had no frothy

fluid animals with profuse thin oedema fluid had very little frothy fluid, as had those with viscid fibrinous oedema fluid. It was often difficult to distinguish between bronchitis stagnation of secretion and haemorrhages into the respiratory tract and lung oedema secretion

Lung weight Pneumonia hypernemia and pulmonary haemorrhages caused an increase of the lung weight but atelectasis did not and animals with white spotted lungs which occurred after overhydration did not have increased lung weights

Only some of those animals that died in uraemia or were killed by intravenous injection of air had increased lung weights after death The position of the body hypernemia at the moment of death overhydration, postmortal pressure in the blood vessels, and clotting time may possibly play a part It was therefore difficult to state normal values for the lung weight in spontaneously dead animals, especially for those that were not examined immediately after death. In association with experimental pulmonary oedema without any noteworthy pneumonia hypernemia pulmonary haemorrhages or postmortal changes (this refers only to non bled animals) the lung weight is a good method for evaluation of the oedema state The presence of pulmonary oedema can be excluded if the postmortal lung weight is low

The water content of the lung In this study the lungs were dried by the heat lamp technique, which has not been used earlier for this purpose. In animals with *haemorrhagic pulmonary* oedema or with pulmonary oedema and marked *hyperaemia or pneumonia* the water content was in some cases normal although the *lung weight*, gross and microscopical observations, X ray and the presence of *frothy fluid* provided evidence of pulmonary oedema. It was difficult to establish "basic values for overhydrated animals without the diagnosis of pulmonary oedema many of them had a water content that exceeded the upper limit of normal range but no other criteria of pulmonary oedema. In spontaneously dead rabbits the water content increased less than the lung weight post mortem except in animals that died markedly overhydrated. Small foci of pulmonary oedema as in the Narkotal series were not evidenced by an increased water content. The method is particularly valuable in the differential diagnosis preferably together with the lung weight, as has been shown by Poulsen (206) in complicated cases further criteria of pulmonary oedema must be satisfied before the diagnosis of pulmonary oedema can be established with a reasonable degree of probability

Microscopical studies There was no *alveolar oedema* in lungs with massive oedema evidenced by all the other criteria notably in the dextran series and in overhydrated rabbits in the adrenaline and the vagotomy series. Microscopical examination was necessary as a means of making the differ

ential diagnosis, especially in the vagotomy series in which some animals had co-existing pulmonary oedema, pneumonia, atelectasis, hyperaemia, and haemorrhages. The diagnosis and quantitation of alveolar oedema of lung-oedema origin was also difficult in the vagotomy "Narkotal" and dextran series, in which alveolar oedema was seen around numerous small areas of haemorrhage and infection.

Only the most severely changed part of the lungs were examined microscopically which means that the evaluation refers to local amounts and is not a general quantitation. In post mortal pulmonary oedema the alveolar oedema fluid was rich in protein, few alveoli contained red cells and alveolar phagocytes, and even peripheral alveoli were well filled.

Microscopical observation is of value in the differential diagnosis rather than in the diagnosis and quantitation of pulmonary oedema.

Lung volume The lung volume was determined in a small number of rabbits. The method was abandoned when it was found that great variations were obtained between the volumes because the air disappeared very quickly from the lung at the least leakage, which occurred easily

The difficulties in establishing which animals have and which have not pulmonary oedema seem to be due to threes factors There is no available diagnostic method which is suitable under any conditions the borderlines are vague many pulmonary processes can co-exist with pulmonary oedema. It is not possible to choose the best method, because the results vary with different forms of lung oedema. On the basis of the observations made in these experiments the range of usefulness of the methods under discussion is outlined.

When three of the six criteria used here are taken as sufficient evidence for pulmonary oedema it may be suspected that pulmonary oedema would be wrongly diagnosed in pneumonia, hyperaemia, and haemorrhages into the lung parenchyma. Moreover quite dissimilar microscopical pictures may be obtained at different combinations of three positive criteria. One rabbit can, for instance have slight uncomplicated pulmonary oedema while another can have pulmonary oedema with evidence of haemorhage. The problem arising in haemorrhages or hyperaemia seems to be a minor one, as abundant haemorrhages are closely associated with haemorrhagic pulmonary oedema, and marked hyperaemia is closely associated with pulmonary oedema. In pneumonia the method failed, however Here, microscopical examination or analysis of the water content was necessary to avoid designating pneumonia as pulmonary oedema.

Poulsen (206) has suggested a combination of water content and lung weight as a means of evaluating and diagnosing pulmonary oedema. This method was tried in the present study and showed good agreement in most cases. The doubtful cases are those in which the presence of both pulmonary oedema and pneumonia (vagotomized animals) pulmonary oedema and hy

peraemia and pulmonary oedema and *haemorrhages* into the lung parenchyma (the dextran series) is suspected In these cases other examinations in addition to lung weight and water *content are necessary notably* microscopical observation in order to establish a reasonably confident diagnosis

These diagnostic difficulties arise not only in experimentally induced pulmonary oedema but also and perhaps more emphatically in clinically *manifest pulmonary oedema It will* be seen in Chapter 2 that clinically acute violent frothy lung oedema is easy to diagnose whereas slowly supervening pulmonary oedema may present great diagnostic difficulties Cases with bronchitis bronchopneumonia atelectasis and/or haemorrhages, in particular are difficult to distinguish *from cases with pulmonary oedema,* and the borderline between hyperaemia and pulmonary oedema is vague In several cases pneumonia or bleeding in the lung parenchyma led to the radiographic diagnosis of fluid retention lung and therapy was thus inadequate Long standing heart failure with pulmonary congestion can *render the radiographic differential* diagnosis difficult Chest radiograph has its greatest value in the diagnosis of fluid retention lung" (uraemic pulmonary oedema) under clinical conditions where overhydration is the main alternative and pneumonia etc. appears less probable (Alwall 1963) The frequency of postmortal pulmonary oedema is high among uraemia patients whether it is final or postmortal *is an open question*

The uremia factor and the fluid factor

Under these experimental conditions, uremia per se seems not to be sufficient to cause pulmonary *oedema other* factors must be present

To find out whether increasing uraemia increases the susceptibility to pulmonary oedema experimental pulmonary *oedema* was induced by four methods in rabbits which had been submitted to bilateral *ureteral ligation.* Increasing uraemia increased the tendency to pulmonary oedema after infusion of dextran and after Narkotal narcosis whether the animals were overhydrated or not this effect of uraemia was insignificant in non-overhydrated vagotomized rabbits and absent after injection of adrenaline, in *overhydrated as well as in non-over* hydrated animals Perhaps severe uraemia increases the susceptibility to pulmonary oedema under certain conditions only or else—hypothetically—some factors of the uraemia phenomena predispose to lung oedema while others counter it (Ascites can be such a factor) Unspecific factors in the experimental conditions can be thought to influence the frequency of pulmonary oedema under otherwise similar conditions

On the basis of these considerations it may be concluded that uraemia following ligation of the ureters can be a factor that under some *conditions in*creases the susceptibility to pulmonary oedema and under other conditions reduces it

The significance of the fluid factor

was studied by overhydration experiments on rabbits whose ureters had been tied, using different solutions for infusion, as well as by overhydration with Ringer's solution in connection with vagotomy Narkotal narcosis, and adrenaline injection. Salt and fluid retention increased the susceptibility to pulmonary oedema after Narkotal' narcosis, adrenaline infection, and vagotomy in anuric rabbits. (Noteworthy in these series was the presence of branchiolitis after Narkotal' narcosis, periarterial interstitial oedema after adrenaline injection and mixed oedema pneumonia, atelectasis, hyperaemia and haemorrhages after vagotomy) Even after administration of blood in amounts that were twice as large as those of the animals circulating blood, slight pulmonary changes developed in only a few cases. But when the plasma volume was increased by the addition of dextran, pulmonary oedema developed in a higher frequency than after the same amount of blood. When the extracellular fluid was increased by infusion of mannitol solution, signs of slight pulmonary oedema arose in only 1 case an increase by infusion of Ringer's solution did not cause pulmonary oedema even at administration of amounts equalling 50—100 % of the body weight, unless the infusion was made too rapidly and the animal was moribund before the infusion When the total fluid was increased by infusion of urea or glucose solution, the animals became very poor but did not develop pulmonary oedema. The toxicity of urea and mannitol solutions rendered the assessment of their oedema producing effect difficult, but this effect seemed to be slight

The main conclusion that can be drawn from these experiments is that overhydration in association with uraemia in rabbits with both their ureters tied produced pulmonary oedema only if it meant an increase of the blood volume. Additional factors are evidently required in order that overhydration by infusion of other solutions shall produce pulmonary oedema. Salt and fluid retention increased the susceptibility to pulmonary oedema after Narkotal' narcosis, adrenaline injection, and vagotomy in anuric rabbits.

The problems that arose in these series may be of special interest, since a parallel can be drawn to clinical conditions where the differential diagnosis against pulmonary oedema presents difficulties in the presence of pneumonia haemorrhages, and atelectasis, etc. (see Alwall [20 21])

Acknowledgements

I wish to express my sincere gratitude to

Professor Nils Alwall for awakening my interest in problems on uraemic pulmonary oedema, for excellent working facilities, for all the support he has given throughout the present investigation and for constructive criticism.

Professor Olle Olsson for help with some of the roentgenological facilities.

Nils O Berg, Prosector at the Institute of Pathology, for generous help and examinations of the histological preparations.

Dr Anders Lunderquist for generous help and examination of the X-ray pictures.

Göran Angmark, fil. kand., the Institute of Statistics, for help with the statistical calculations.

Mrs. Ilga Priedits for skilful technical assistance, Mrs. Maria Roth for conscientious secreterial work, Karl Rähesoo Pharm. D and Mr Jozsef Szalai, for careful work with the diagrams, Miss Mona Frisell for the histological preparations, Miss Ing Britt Augrell for the photographs, Boryslaw Kurowski, jur mag, Miss Judith Csöreg, Mrs. Maria Kurowska for technical assistance, Miss Elsa Schmitz for helping with proof reading and Mr A. Nilsson for good care of the animals.

The investigation was supported by grants from the Medical Faculty of the University at Lund, Sweden and (through professor Nils Alwall) from the Swedish Medical Research Council and Marie-Louise Schéle's Memorial Fund.

References

1 *Aikawa, J. K.* Fluid volumes and electrolyte concentrations in normal rabbits. Amer. J. Physiol. *162* 695, 1950.

2. *Alexander, S., Eat n, J. C., Freedman, H. J.* Some experimental observations on the action of intravenous hypertonic urea in dogs, with particular reference to plasma volume and tissue urea changes. J. Neurol. Neurosurg. Psychiat. 24 148, 1961.

3 *Altschule M. D.* Acute pulmonary edema. Grune & Stratton, New York, 1954.

4 *Altschul M. D. Gilligan, D. R.* The effects on the cardiovascular system of fluids administered intravenously in man. II. The dynamics of the circulation. J. clin. Invest. 17 401 1938.

5 *Alwall N.* On the artificial kidney I. Apparatus for dialysis of blood in vivo. Acta med. scand. *128* 317 1947

6 *Alwall N.* Treatment of acute renal failure with anuria-oliguria. The importance of weight control and the hazards of excessive electrolyte-fluid supply. Nord. Med. *40* 2378, 1948.

7 *Alwall, N.* On the artificial kidney II. Some clinical experiences and problems in the treatment of uremia. Nord. Med. 41 411 1949.

8 *Alwall N. Bergstrom, B. Gedda, P. Norviit L., Steins A.* On the artificial kidney IV (Technique in animal experiments) Acta med. scand. *132* 392, 1949.

9 *Alwall N. Norviit L. Steins A.* On the artificial kidney V. Some experiences during the study of dialytic treatment on animals with uremia caused by mercuric chloride poisoning. Acta med. scand. *132* 477 1949

10. *Alwall N. Hermer B.* On the artificial kidney VI. Some views on the indications for treatment of uremia and for active removal of oedema by means of our artificial kidney *based on studies of uremic* material not treated with this method. Acta med. scand. *132* 572, 1949

11 *Alwall N.* On the artificial kidney VIII. Cases of acute renal failure with anuria-oliguria treated with (1) Excessive fluid-electrolyte supply (2) Fluid supply under weight control to avoid or reduce fluid retention-oedema (3) Artificial kidney. Acta med. scand. Suppl. 229 1, 1949.

12 *Alwall N.* On the artificial kidney XIII. Construction details of the dialyser-ultrafiltrator intended for homo. Acta med. scand. Suppl. 229 30 1949.

13. *Alwall N.* Treatment of uremia, soporific poisoning and life-menacing electrolyte-fluid retention, especially by means of artificial kidney. Urologia, (Milano) 2[illegible] 482, 1952.

14. *Alwall, N. Lunderquist A., Tornberg A.:* Four cases of acute glomerulonephritis anuric for 14, 21 42 and 44 days respectively. Nord. Med. 48 1665 1952.

15. *Alwall, N.* A weighing machine for bed patient. An indispensable item of equipment for every hospital. Acta med. scand. 143 158, 1952.

16. *Alwall, N. Lunderquist A., Olsson, O.:* Studies on electrolyte-fluid retention. I. Uremic lung-fluid lung? On pathogenesis and therapy (A preliminary report.) Acta med. scand. *146* 157 1953.

Acknowledgements

I wish to express my sincere gratitude to

Professor Nils Alwall for awakening my interest in problems on "uraemic pulmonary oedema" for excellent working facilities, for all the support he has given *throughout the present investigation* and for constructive criticism

Professor Olle Olsson for help with some of the roentgenological facilities

Nils O Berg Prosector at the Institute of Pathology for generous help and examinations of the histological preparations

Dr Anders Lunderquist for generous help and examination of the X ray pictures

Göran Angmark fil. kand., the Institute of Statistics for help with the statistical calculations

Mrs. Ilga Priedits for skilful technical assistance, Mrs. Maria Roth for conscientious secreterial work, Karl Rähesoo, Pharm D and Mr Jozsef Szalai for careful work with the diagrams, Miss Mona *Frisell for the histological preparations*, Miss Ing Britt Augrell for the photographs, Bozyslaw Kurowski jur mag Miss Judith Csöreg, Mrs. Maria Kurowska for technical assistance, Miss Elsa Schmitz for helping with proof reading and Mr A. Nilsson for good care of the animals

The investigation was supported by grants from the Medical Faculty of the University at Lund Sweden and (through professor Nils Alwall) from the Swedish Medical Research Council and Marie-Louise Schéle s Memorial Fund.

References

1 Allison, J. K. Fluid volumes and electrolyte concentrations in normal rabbits Amer J Physiol. 162 685, 1950.

2 Alexander S., Eaton, J. C., Freedman, H J. Some experimental observations on the section of intravenous hypertonic urea in dogs, with particular reference to plasma volume and tissue urea changes. J Neurol. Neurosurg. Psychiat. 24 148, 1961

3 Altschule M D. Acute pulmonary edema. Grune & Stratton, New York, 1954.

4 Altschul M D Gilligan, D R. The effects on the cardiovascular system of fluids administered intravenously in man. II The dynamics of the circulation J clin. Invest 17 401 1938.

5 Alwall N On the artificial kidney I. Apparatus for dialysis of blood in vivo. Acta med scand 128 317 1947

6 Alwall N Treatment of acute renal failure with anuria-oliguria The importance of weight control and the hazards of excess electrolyte-fluid supply Nord. Med 40 2378, 1948

7 Alwall, N On the artificial kidney II Some clinical experiences and problems the treatment of uremia. Nord. Med. 41 411, 1949.

8 Alwall, N. Bergsten, B Gedda, P Norviit L Steins, A. On the artificial kidney IV (Technique in animal experiments) Acta med. scand. 132 392, 1949.

9 Alwall N Norvit L Steins, A. On the artificial kidney V Some experiences during the study of dialytic treatment on patients with uremia caused by mercuric chloride poisoning Acta med. scand. 132. 477 1949

10 Alwall N. Herner B. On the artificial kidney VI. Some views on the indications for treatment of uremia and for active removal of oedema by means of our artificial kidney based on studies of uremic material not treated with this method. Acta med. scand. 132: 578, 1949.

11 Alwall N. On the artificial kidney VIII Cases of acute renal failure with anuria-oliguria treated with (1) Excessive fluid-electrolyte supply (2) Fluid supply under weight control to aid or reduce fluid retention-oedema. (3) Artificial kidney Acta med. scand. Suppl. 229 1 1949

12. Alwall, N. On the artificial kidney XIII. Construction details of the dialyser-ultrafiltrator intended for home. Acta med. scand. Suppl. 229 30 1949

13 Alwall N Treatment of uremia, soporific poisoning and life menacing electrolyte-fluid retention, especially by means of artificial kidney Urologia, (Milano) 2 483 1952

14. Alwall, N Lunderquist A., Tornberg A. Four cases of acute glomerulonephritis anuric for 14, 21, 42 and 44 days respectively Nord Med. 48: 1465, 1952.

15. Alwall N A weighing machine for bed patients. An indispensable item of equipment for every hospital. Acta med. scand. 143 144, 1952.

16. Alwall N Lunderquist, A., Olsson, O Studies on electrolyte-fluid retention. I. Uremic lung-fluid lung? On pathogenesis and therapy (A preliminary report.) Acta med. scand. 146 157 1953.

Acknowledgements

I wish to express my sincere gratitude to

Professor Nils Alwall for awakening my interest in problems on "uraemic pulmonary oedema", for excellent working facilities, for all the support he has given throughout the present investigation and for constructive criticism.

Professor Olle Olsson for help with some of the roentgenological facilities.

Nils O. Berg, Prosector at the Institute of Pathology, for generous help and examinations of the histological preparations.

Dr. Anders Lunderquist for generous help and examination of the X-ray pictures.

Göran Angsmark, fil. kand., the Institute of Statistics, for help with the statistical calculations.

Mrs. Ilga Priedits for skilful technical assistance, Mrs. Maria Roth for conscientious secreterial work, Karl Råhesoo, Pharm. D. and Mr. Jozsef Szalai for careful work with the diagrams, Miss Mona Frisell for the histological preparations, Miss Ing-Britt Augrell for the photographs, Bozyslaw Kurowski, jur. mag., Miss Judith Csöreg, Mrs. Maria Kurowska for technical assistance, Miss Elsa Schmitz for helping with proof reading and Mr. A. Nilsson for good care of the animals.

The investigation was supported by grants from the Medical Faculty of the University at Lund, Sweden and (through professor Nils Alwall) from the Swedish Medical Research Council and Marie-Louise Schéle's Memorial Fund.

45. *Carr C. J. M aser R. Schmidt J. E., Krant J. C.* The f te of mannitol and mannitan in the animal body J. biol. Chem. *102* 721 1933.

46. *Cheng K. K.* Studies on the mechanisms of adrenaline lung oedema. J. Path. Bact. *76* 211 1938.

47. *Cohnheim, J. Lichtheim, L.:* Uber hyd ä-mi und hydrämisches Oedem. Arch. f path. Anat. und Phys. *69* 106, 1877

48. *Courtice F. C.* Pulmonary edema. Clinical implications of laboratory experiments. Aust. N. Z. J. Surg *22* 177 1933

49. *Courtice F. C., Korner P. I.* The effect of anoxia on pulmonary oedema produced by massiv intravenous infusions. Aust. J. exp. Biol. med. Sci. *30* 511 1952.

50. *Creevy C. D.* Observations on hemolysis during transurethral resection The ffects of urea Trans. Amer Ass. gen. urin. Surg *43* 8, 1951

51. *Conleng G. Varga, L., Szam I.* Die röntgenologische Darstellung des experimentellen Lungenödem. Z. Kreisl. Forsch. *49* 990 1960.

52. *Daniel R. A., Cat. B. H.* W t lung- an experimental study Ann. Surg. *127* 836, 1948.

53. *Dastre M. Loye P.* Le la age du sang. Arch de physiol norm et path p 93, 1888

54. *Dewey C. E.* Heart failure in acute nephritis Quart J Med *78* 163, 1931

55. *Degl J. T. Wilson, J. S., Est s, E. H. Warren, J. V.* The effect of intra enous inf sion of physiologic saline solution on the pulmonary arterial and pulmonary capillary pressure in man. J. clin. In est *30* 345, 1951

56. *De Fazio V. Christensen, R. C. Regan, T. J. Due J. J. Verda, Y. Hell me H. K.* Circulatory changes in acute glomerulonephritis Circulation, *20* 190, 1959.

57. *De Gowin, E. L.* Gra sequelae of blood transfusions A. I ical study of 13 cases occurring in 3500 blood transfusion Ann Intern Med *11* 1 77 1938

58. *De Gowin E. L. Hard n, R. C. Usener J. R.* Blood tr nsfusions W. B. Saunders Co Philadelphia, London 1949

59. *De Graeff J. d. Deen, P.:* Bilateral renal cortical necrosis. Acta med. scand. *165* 341, 1959

60. *De Pass S. W. Stein, J. Poppel, M. H.:* Pulmonary congestion and edema in uremia. J. Amer med Ass. *162* 5, 1956.

61. *Deck, W.:* The evil sequelae of complet bed rest. J. Amer med. A s. *125* 1003, 1944.

62. *Dominiquez R., Corcoran, A. C., Page I. H.* Mannitol. Kinetics of distribution, excretion and utilization in human beings. J. Lab. clin. Med. *32* 1192, 1947

63. *D niech, I.* Uremic edema of the lungs. Am. J. Roentgenol. *58* 620, 1947

64. *Doniach, I.* Uraemic oedema of the lungs. Lancet 2 911 1949

65. *Doniach I. Morri on, B., Steiner R. E.:* Lung changes during hexamethonium therapy for hypertension. Brit. Heart J *16.* 101 1954

66. Dorland' Illustrated Medical Dictionary W. B. Saunders Company Philadelphia and London, 1957

67. *Domar J. W.* The problem of overtransfusion in massiv hemorrhage Ann. Surg *148* 73, 1958.

68. *D enckhahn, F. O.* Die Beziehung zwischen Pulmonalvenendruck und Ödementstehung beim experimentellen Adrenalinlungödem Verh. dtsch. Ges. Kreisl. Forsch. *23* 372, 1957

69. *Drenckhahn, F. O.* Die Ödementstehung beim experimentellen Adrenalinlungenödem. Pflügers Arch. f. Physiol. *266* 231 1958

70. *Drinker C. K.* Pulmonary edema and inflammation Harvard Univ Press. Cambridge Mass. 1947

71. *Drinker C. K. Warren, M. F.* The genesis and resolution of pulmonary transudates and exudates. J. Amer med. Ass. *122* 200 1943.

72. *Dundee J. W. Richards, R. R.* Effect f azotemia upon the action f intra enou barbiturate anesthesia. Anesthesiology *15* 333, 1954

73. *D ischer S. H. Benfield W. C. B rymes A. D.* Post-mortem pulmonary edema. Yale J. Biol. Med. *22* 565, 1950.

17 *Alwall N* Treatment of electrolyte-fluid retention by ultrafiltration of the blood in vivo The Kidney Ciba Foundation, London, p 221 1954

18. *Alwall N.* Ergebnisse von 700 Behandlungen mit der Künstlichen Niere (Dialyse Ultrafiltration) während der Jahre 1946—195 sowie Mobilisierung, Tracheotomie, Respirationsbehandlung u.s.w und röntgenologische Kontrolle der Flüssigkeitsgleichgewichts. Dtsch. med. Wschr *83* 930 1008 1958.

19 *Alwall N* Fifteen hundred treatments with the artificial kidney (dialysis, ultrafiltration) 1946—1960 Proc 1st. Int Congr Nephrol Genève/Evian, p 332 1960

20 *Alwall N. Fluid lung in anuria-oliguria.* A study of 607 cases. Proc. 6th Internat. Congr Int Med. p 10 1960

21 *Alwall N* Therapeutic and diagnostic problems in severe renal failure Scandinavian University Books Stockholm, 1963

22. *Anthonisen P* Hjert ts minutvolumen ved uremi. Nord Med *64* 1053 1960

23. *Anthonisen, P Holst E* Determinati n of cardiac output and other hemodynamic data in uremic patients using dye dilution technique Scand J Clin. Lab Invest. *12* 481 1960.

24 *Antila V Härtel C* Über die Wirkung von Palfium a f da Adr nalin Lungenödem der Mau Ann. Med. exp Fenn. *37* 259 1959

25. *Auer J Gates F L.* Experiments on the causation and amelioration of drenalin pulmonary edema. J exp Med. *20* 201 1917

26. *Barden R. Co per D* The roentgen appearance of the chest in diseases f fecting the periph ral vascular ystem of the lungs. Radiology *51* 44 1948.

27 *B rletg M A hl D* Action comparée des différent groupes de ynaptoplegiques dans les oedemes pulm n ires expérimentaux d origi barbiturique t adrenalique Therapi *8* 801 1953

28 *Barry K G Be man L R M* itol infusion III The acute effect f the intravenous infusion of mannit l on blood and plasma volumes. New Engl. J Med. 264 1085 1961

29 *Barry K C Malloy J P* Oliguric renal failure Evaluation and therapy by the intravenous infusion of mannitol. J Amer med. Ass *179* 510 1962.

30 *Baruch A. L.* The use of helium in the treatment of asthma and obstructive lesions in the larynx and trachea. Am. J t. Med. *9* 739 1935.

31 *Bass H E Greenberg D Singer E, Miller M A.* Pulmonary changes in uremia. J Amer med. Ass. *148* 724 1952.

32 *Bass H E. Singer E.* Pulmonary changes in uremia. J Amer med. Ass. *144* 819 1950

33 *Battelli F Taramasi P* Toxicité de la substance active des capsules surrénales. C. R. Soc. Biol. Paris *54* 815 1902

34 *Bergman F* To be published 1964

35. *Bernard C.* Leçons sur la physiologie et la pathologie de système nerveux Baillière et Fils, Paris *2* 344 1858.

36. *Bernstein, L. M Blumberg B Arkin M C* Osmotic diuretic treatment f refractory edema. Circulati n *17* 1013, 1958.

37 *Bluemle L. W Webster C D Elkinton, J R* Acut tubular necrosis. Arch. intern. Med *104* 180 1959

38 *Borgström, K E., Ising I Linde E Landerquist A* Experimental pulmonary edema Acta Radiologica *54* 97 1960

39 *B rgström, K E. Landerquist* A Pulmonary edema. Encyclopedia of Medical Radiology Band IX. Springe Verlag. Heidelberg In press 1963.

40 *Born G V R* Acute edema in th i lated perf sed lung rabbits. J Physiol. *124* 502, 1954

41 *Bouchard C Claude H* Recherches perimentales u l d enaline C. R Ac Sc. 135 928, 1902.

42. *Braun, F* Experimentelles zum Lungenödem. Wien klin. Wschr *46* 202, 1933.

43. *Cameron C R* Pulm nary oedema Brit Med J *1* 965 1948.

44. *Carr C. J Krant J C.* S gar alcohols XII The fate of poly galitol nd man itol in the animal body J biol. Chem *121* 221 1938.

tinal dropsy. Guy's Hosp. Rep. (3rd series) 24 153, 1879.

102. *Goodpasture E. W.:* The significance of certain pulmonary lesion in relation to the etiology of influenza. Amer J med. Sci. 158 863, 1919.

103. *Goodrich W A.:* Pulmonary edema. A correlation of X-ray appearance and physiological changes. Radiology 51:58, 1948.

104. *Gordh, T.:* Postural circulatory and respiratory changes during ether and intravenous anaesthesia. Acta chir scand. 91 Supplement 102, 1945.

105. *Gould D. M Terrence D J* Pulmonary edema. Am. J Roentgenol. 73 366, 1955.

106. *Gouley B. A., Eiman J.:* The pathology of rheumatic pneumonia. Am. J med. Sci. 183 359 1932.

107. *Gowdey C. W. Hatcher J D Sunahara, F A* Cardiovascular responses in dogs to large intravenous infusions. Canad. J Biochem. Physiol 42 282, 1964

108. *Grollman, E F Grollman, A.* Toxicity of urea and it role in the pathogenesis of uremia. J clin. Invest. 38 749, 1959

109. *Grönwall A.:* Dextran and its use in colloidal infusion solutions. Almquist & Wiksell, Stockholm, Sweden, 1957

110. *Grönwall, A Ingelman, B.:* Untersuchungen über Dextran und sein Verhalten bei parenteraler Zufuhr II Acta Physiol. scand. 9 1 1945

111. *Haddy F J Campbell, G. S Visscher M B* Pulmonary vascular pressures in relation to edema production by airway resistance and plethora in dogs. Amer J Physiol. 161 336, 1950

112. *Hall A.* Memoire sur les parties sensibles et irritables du corps animal. Lausanne. 1 221 1756—1760.

113. *Halpern, B N Cruchard S.:* Prevention de l'oedème aigu du poumon expérimental par les antihistaminiques de synthèse. C. R Soc Biol. Paris. 141 1038, 1947

114. *Halpern,* B N *Cruchard S., Vermeil G., Roux J L.* Etude pathogénique et thérapeutique de l'oedème aigu du poumon expérimental. Arch. int. Pharmacodyn 82. 423, 1950.

115. *Harington, M Kincaid-Smith, P M McMichael J.:* Results of treatment in malignant hypertension. Brit. Med. J 2 969 1959

116. *Harrison, W Liebow A. A.:* The effects of increased intracranial pressure on the pulmonary circulation in relation to pulmonary edema. Circulation 5 824, 1952

117. *Harrison, W Liebow A. A.:* The effects of massive infusion with special reference to pulmonary congestion and edema. Yale J Biol. Med. 26 372, 1954.

118. *Helmers, R.:* La pneumopathie urémique. Acta clin. belg. 14 463, 1959

119. *Henkin, R. I Bish p H A.:* Pulmonary clearance of radioactive iodinated serum albumin in man. J Lab. clin. Med. 60 700 1962.

120. *Henkin, R. I Maxwell M H Murray J F* Uremic pneumonitis. A clinical, physiological tudy Ann. intern. Med. 57 1001 1962.

121. *Hennemann, P H* Acute pulmonary edema with special reference t experimental studies. New Engl. J Med. 235 590 619 1946.

122. *H renkeiser G Z* Röntgendiagnostik des Lungenödems. Fortschr Röntgenstr 89 135, 1958.

123. *Henschel, G.:* Die urämische Lunge. Z. ges Inn. Med. 3 62, 1958.

124. *Hild en, T Kragsgaard A. R Wiastrup B F* tal pulmonary changes during the medical treatment of malignant hypertension. Lancet 1 830, 1958.

125. *Hod an, C J.:* Pulmonary oedema and batswing shadow J Fac. Radiol. (Lond.) 1 176, 1950.

126. *H ppe H C., Wissler R W* Uremic pneumonitis. Amer Path. 31 361 1955.

127. *Houck G. R.:* Disappearance of mannitol and PAH from plasma of bilaterally nephrectomized dogs. Amer J Physiol. 165 102, 1951

128. *Howard J M Fremby J P Artz, P Sako, Y* The f te of dextran and modified gelatine in casualties with renal insufficiency. Surg. Gynec. Obstet. 100 207 1961.

129. *H leber W Caeten, G., Harrison, T R.* Experimental hypervolemic heart fail-

74 *Eaton R M.*, Pulmonary edema. Experimental *observations on dogs following* acute peripheral blood loss. J Thorac. Surg *16* 668, 1947

75 *Ehrich W McIntosh J F* The pathogenesis of bronchiolitis obliterans. Arch. Path. *13* 69 1932

76 *Eichna L. W Farber S J., Berger A R Rader B Smith, W W Albert R E.*, Non-cardiac circulatory congestion simulating congestive heart failure. Trans. Ass. Amer Physcns. 67 72, 1954

77 *Elkinton, J R.* Non utilization of mannitol in uremic patients. Fed. Proc. *8* 41 *1949*

78. *Elliot T R* The action of adrenaline. J Physiol Lond. *32* 401 *1905*

79 *Ellis A. W M.*: Heart failure in acute nephritis. Quart. J Med. *5* 533, 1936.

80 *Emerson, H* Artificial respiration in the treatment of edema of the lungs. Arch intern. Med. *3* 368 1909

81 *Erlanson P Lindholm, T Lindqvist B Svensson, A* Artificial respiration in severe renal failure with pulmonary insufficiency Acta med. scand. *166* 81 1960

82 *Farber S* Studies on pulmonary edema. I. The consequences of *bilateral cervical* vagotomy in the rabbit. J exp. Med. *66 397 1937*

83 *Farber S* Studies on pulmonary edema. II The pathogenesis of neuropathic pulmonary edema. *J* exp Med. *66* 405 1937

84 *Farber S* Neuropathic pulmonary edema. Arch. Path. *30* 180 1940

85 *Fishberg A M* Advances in the treatment of renal disease. Practitioner *175* 408, 1955

86. *Fowler N O Bloom, W L., Ward J A., Franch R H* Hemodynamic effects of *hypervolemia with and without anemia.* Circulation *14* 937 1956.

87 *F wler N O Bloom W L., Ward J A.* Hemodynamic ffects of hypervolemia with and without anemia. Circulation Res *6* 163, 1958.

88. *Fremont-Smith, F Forbes H S.*, Intra ocular and intra-cranial pressure. Arch. Neurol. Psychiat. (Chic.) *18* 550 1927

89 *Frerichs F T.*, Die Bright sche Nierenkrankheit und deren Behandlung. Braunschweig 1851

90 *Frey O* Die pathologischen Lungenveränderungen nach Lähmung der Nervi vagi. *Wilhelm Engelmann, Leipzig 1877*

91 *Friedberg C. K* Congestive heart failure of renal origin. Amer J Med. *9* 164, 1950

92. *Fritz, H Lindqvist B.*, Pulmonary ventilation (Becklake s index) in renal failure, with special reference to overhydrated patients with "Fluid lung Acta med. scand. *169* 181 1961

93. *Funck Brentano J L. Lossky-Nekhorocheff I Altman J* Étude de *expérimentale des manifestations cérébrales de l'in*toxication pour l'eau. Rev franc. d'études Clin. Biol. *4* 46, 1959

94 *Földi M Koramy A., Szabo G* Über die Entst hung anämischer Ödeme. Acta med. scand. *129* 486 1948.

95 *Ghalioungui P.*, Medicine in the days of the pharaos. Ciba Symp. *9* 206 1961.

96. *Gibbon, J H Gibbon, M H* Experimental pulmonary edema *following lobecto*my and plasma infusion. Surgery p. 694, 1942

97 *Gibbon, J H Gibbon, M H Kraul C W* Experimental pulmonary edema following lobectomy and blood transfusion. J thorac. Surg *12* 60 1942.

98. *Gilligan R Altschule M D Volk M C* The effects on the cardiovascular system *of fluids administered* intravenously in man. I. Studies of the amount and duration of changes in blood volume J clin. Invest. *17* 7 1938.

99 *Glass A* Über Beeinflussung des Adrenalins — Lungenödems durch experimentelle Verletzungen des Hirnstammes und des *Sympathikus. Arch. exp* Path. Pharmak. *136* 88, 1928.

100 *Goldenberg M Crane R. D Popper H* Effect of intravenous administration of dextran a macromolecular ca bohydrate in animals. Am. J Clin. Path. *17* 939 1947

101 *Goodhart J F On cut* dilatation of the heart as cause of death in scarla-

tical dropsy Guy Hosp. Rep. (3rd series) 24 153, 1879.

102. *Goodpasture E. W.:* The significance of certain pulmonary lesions in relation t the etiology of influenza. Amer J med. Sci. 158/863, 1919

103. Goodrick, *W A.*, Pulmonary edema. A correlation of X-ray appearance and phy siological changes. Radiology 51 58, 1948.

104. *Gordh, T* Postural circulatory and respiratory changes during ether and intravenous anesthesia. Acta chir scand. 92 Supplement 102, 1945.

105. *Gould, D M Torrance D J* Pulmonary edema. Am. J Roentgenol. 73 366, 1955.

106. *Goulry B. A., Elman, J* The pathology of rheumatic pneumonia. Am. J med. Sci. 183. 350, 1932.

107 *Gowdey C. W Hatcher J D Sunahara F A.* Cardiovascular responses in dogs to large intravenous infusions. Canad. J Biochem. Physiol. 32 282, 1954.

108. *Grollman, E F Grollman, A.:* Toxicity of urea and it role in the pathogenesis of uremia. J clin Invest. 38 749 1950

109. *Grönwall, A* Dextran and it use in colloidal infusion solutions. Almqvist & Wiksell, Stockholm, Sweden, 1957

110. *Grönwall, A Ingelman, B.*, Untersuchungen über Dextran und sein Verhalten bei parenteraler Zufuhr II Acta Physiol. scand. 9 1 1945

111. *Haddy F J Campbell O S., Visscher M B.*, Pulmonary vascular pressures in relation to edema production by airway resistance nd plethora in dogs. Amer J Physiol. 161 336, 1950.

112 Haller A Memoire sur les parties sensibles et irritables du corps animal. Lausanne. 1 224, 1756—1760.

113 *Halpern, B. N Cruchaud S.*, Prevention de l'oedème aigué du poumon expérimental par les antihistaminiques de synthèse. C R. Soc. Biol Paris. 141 1038, 1947

114. *Halpern, B N Cruchaud S Vermeil G., Roux J L.* Etude pathogénique et thérapeutique de l'oedème aigué d poumon experimental. Arch. int. Pharmacodyn 85 435, 1958.

115. *Harington, M Kincaid-Smith, P McMichael J.:* Results of treatment in malignant hypertension. Brit. Med. J 2 969 1959

116. *Harrison, W Liebow A. A.:* The effects of increased intracranial pressure on the pulmonary circulation in relation to pulmonary edema. Circulation 5 824, 1952.

117 *Harrison, W Liebow A. A.:* The effects of nasal infusion with special reference to pulmonary congestion and edema. Y le J Biol. Med. 26 372, 1954.

118. *Helmans, R.*, La pneumopathie uremique. Acta clin. belg. 14 403 1959.

119 *Henkin, R. I Bishop, H A.:* Pulmonary clearance of radioactive iodinated serum albumin in man. J Lab. clin. Med. 60 709, 1962.

120. *Henkin, R. I Maxwell M H Murray J F* Uremic pneumonitis. A clinical, physiological study Ann. intern. Med. 57 1001 1962.

121. *Hennemann, P H* Acute pulmonary edema with special reference t experimental studies. New Engl. J Med. 235 590 619 1946

122. *Herrnheiser G* Zur Röntgendiagnostik des Lungenödems Fortschr Röntgenstr 89 125, 1958.

123 *Heuchel G* Die urämische Lunge. Z. ges. inn Med. 2 82, 1946.

124. *Hildeen, T Kregsgaard A. R Wimtrup B* Fatal pulmonary changes during the medical treatment of malignant hypertension. Lancet 1 830, 1958.

125 *Hodson, C. J* Pulmonary oedema and batswing shadow J Fac. Radiol. (Lond.) 1 176, 1950

126 *H pps H C., Wissler R. W* Uremic pneumonitis. Amer Path. 31 261, 1955.

127 *Houck, G. R* Disappearance of mannitol and PAH from plasma of bilaterally nephrectomized dogs. Amer J Physiol. 165 102, 1951.

128 *Howard J M Fromby J P Artz, P Sako, Y* The fate of dextran and modified gelatine in casualties with renal insufficiency Surg. Gynec. Obstet. 100 207 1961

129 *Huckabee W Casten, G., Harrison, T R* Experimental hypervolemic heart fail-

ure Its bearing on certain general principles of heart failure Circulation I 343 1950

130 Ikkos D Luft R Kroppens extracellulära vätskerum. Nord Med. 57 13 1957

131 Ivanitskaya M A Roentgen picture of nephrogenic edema of lungs. Klin. Med. (Mosk.) 21 54 1943.

132 Jackson F The radiology of acute pulmonary oedema. Brit. Heart J 13 503, 1951

133. Javid M Urea—new use of an old agent. Surg. Clin. N Amer 907 1958.

134. Javid M Anderson, J The effect of urea on cerebrospinal fluid pressure in monkey before and after bilateral nephrectomy J Lab. clin. Med. 53 484 1959

135 Javid M Settlage P Effect of urea on cerebrospinal fluid pressure in human subjects. J Amer med Ass. 160 943 1956.

136. Jepsen, O L., Lindhardtsen, F Röntgenforandringar ved akut lungedem. Fremkaldt ved transfusionsbehandling. Ugeskr Læg. 124 827 1962.

137 Johnson S Expérimental production and prevention of acute edema of the lungs in rabbits. Proc. Soc. exp. Bi L (N Y) 25 181 1927

138. Josue O Bloch, L. Oedème aigu du poumon expérimental par injection intraveineuse d xtraits d couche corticale de capsules surrénales. Bull. Soc Méd. Paris. 46 55 1908.

139 Jung S Zucht und Haltung der wichtigsten Laboratoriumsvers chstiere VEB Gustn Fisch r V lag Jena 1958

140 Kabanchik M Kleimans V Pulm n uremico. Pren. méd argent. 38 2143 1951

141 Kasanen, A Lahesmaa R The effect of blood transfusion and fluid therapy on the ital capacity and blood circulati n tim in uremic pati nts. Acta med scand 172 555 1962

142 Kassire J P Schwart W B Acut gl merulonephritis. N w Engl. J Med. 265 686, 1961

143. Keyriläinen, T O Pamsonen, M K Pulmonary edema produced by cervical vagotomy Ann. Med exp Penn 36 87 1958.

144 Khomiakov I C Kainova S S X-ray diagnosis of nephrogenic pulmonary edema Urologlia 26 17 1961

145. Klima, R Rosegger H Eigenartige Krankenheitsbilder zufolge von Lungenödem bei Niereninsuffiziens. Med. Klin. 32 85, 1936.

146. Knoll P. Bemerkungen zur Infusion blutwarmer physiologischer Kochsalzlösung in das Gefässystem. Arch. exp. Path. Pharmak. 36 293 1895

147 Koenig H Koenig R Studies on the pathogenesis of ammonium pulmonary edema. Amer J Physiol. 158 1 1949

148. Kohler D Barb M Variation des liquides du poumon dans l'oedème pulmonaire aigu d origine barbiturique Comparaison avec l oedème adrénalique. C. R. Biol. (Paris) 148 299 1954

149 Korner P L. The effect of n radrenaline—induced systemic vasoconstriction on the formation of pulmonary edema Aust. J e p. Biol. med. Sci. 31 405, 1953

150 Kraus F Über Lungenödem. Z. xp. P th. Ther 14 402 1913

151 Krumbhaa E B Chanutin A. Studies n experimental plethora in dogs and rabbits. J exp Med. 35 847 1922.

152. Lagrang E P mel R Recherches perimentales ur l oedème pulmonaire Arch. Int. Pharmacodyn, 9 445 1949

153 Land A M Gutting R A Larson P S. The size of the extracellular fluid compartment before and after massive infusions. Amer J Physiol. 130 421 1940

154 Landsteine K Über Agglutinationserscheinungen normal n menschlich n Blutes. Wien. klin. Wschr 14 1132, 1901

155. Langendorf R P ck A Elektrokardiogramm bei akute nephritis. Acta med. scand 94 1 1938.

156. Last J M M D nald G O Jane R A Bond E. E Rat of equilibration of l uli and man lt l between plasma and interstitial water i edematous tates. J Lab. clin. Med. 39 62 1952.

157 Lazaris J L, S rebro skaya, I A The

pathogenesi of pulmonary edema. Sovets. Med 24 97 1960

158 *Legallois M* Experiences sur le principe de la vie p 212 D'Hautel, Paris 1812.

159 *Legendre* Quoted from Luisada, 1940

160 *Lelong, M Bernard J* L'image radiologique d'un cas d'oedème aigu du poumon d'origine rénale chez un enf nt Soc. Med Hôp. Paris 177 185 1937

161 *Lepeschkin, E.:* Das Elektrokardiogram Verlag Theodor Steinkopff Dresden, 1937

162. *Levine H D* Spontaneous changes in the normal rabbit electrocardiogram Amer Heart J 24 209 1942.

163 *Lerow P Sigstad H* Glomerulonefritis with initial lung purpura. Act med. scand. 168 405, 1960.

164. *Lorber V* Lung edema following bilateral vagotomy J exp Med 70 117 1939

165. *Lower R.* Tractat de Corde, London. 1669. (Quoted from Short 1944)

166 *Lower R.* Philosoph Trans. Royal Soc. London, 1 128 1665—1666 (Quoted from Short 1944)

167 *Luisada A.:* Beitrag zur Pathogenese und Therapie des Lungödems und des Asthma cardiale Arch exp Path. Pharmak. 132 313, 1928

168 *Luisada, A.* The pathogenesis of par xysmal pulmonary edema Medicine 19 475, 1940.

169 *Luisada, A* Therapy of paroxysmal pulmonary edema by antifoaming agents Circulation 2 872, 1950

170 *Luisada A., Sarnoff S J* Paroxysmal pulmonary edema consequent t stimulation of cardiovascular receptors I II III Am Heart J 31 270, 1946

171 *Lunderquist* A (I collaboration ith N Alwall, A Tornberg) On the artificial Kidney XXI The efficacy of the dialyser —ultrafilter intended for human use, including preliminary report on treatment of oedemac patient by means of ultrafiltration Acta med. scand 143 307 1952

171 *Lund rquist A* Konstgjord njure. VIII. Ultrafiltrationsbehandling id experimentell Ödem hos kanin. Nord Med 50 1209 1953

172. *Läwen M* Über die Entstehung des Lungödems. Beitr path. Anat. 14 401 1893.

173. *MacKeen, A D* Symposium on oedema. III. Review of the patho-physiology of pulmonary oedema. N S. med. Bull. 29 232, 1950.

174 *Macht D L., Ting G C.:* The ffect of some polyhydric lcohol on the beha iour of rats in the circular maze Amer Physiol. 60 496, 1922.

175. *Maleti* Quoted from Luisada 1940.

176. *Manery J F Hastings A B* The distribution of electrolytes in mammalian tissues. J Biol. Chem. 127 657 1939

177 *M rx G. F Koenung J W O Lin, L. R.:* Dilutional hypers lemia during transurethral resection of the prostate. J Amer med. Ass. 174 1834 1960.

178 *Marx G F Rothman R., O Lin, L. R* Intra enous infusions of 10 per cent cytol, 5 per cent dextrose/water or dextra in dogs. J Urol (Baltimore) 84 424, 1960

179 *M person, S Patt rson, R. M M ker A Le Brie S. J Mayerson, P* Permeability of lymphatic essels Amer J Physiol. 203 98, 1962.

180 *Mend nhall R. M Remarine P M Geral B* W ter sodium and potassium content of human, guinea pig and rabbit lung Proc. Soc exp Biol. 82 318, 1953.

181 *M Lien, P* Oedema pulmonaire et uremie C. R. XIII. Congr I ternat. Med. Paris 4 219, 1900.

182. *M rrill J P Smith S., Callahan, E. F Thorn, G U* The use of an artificial kidney II. Cl nical experiences J clin. Investig. 29 425, 1950

183 *Miller J Matthews, S. A.* A stud of the mechanical factors in experimental acute pulmonary edema Intern. Int Med 4 356 1909

184 *Moll n, P L.* Blood transfusion in clinical medicine Blackwell Scientific Publications, Oxford 1961

185 *Moon, V H Morgan, D R.:* Experimental pulmonary edema. Arch. Path 21 565, 1936

186. *Morita, Y* Hemodynamic and lectrolyte studies in acute glomerulonephritis. Clin. Res. Proc 5 206, 1957

ure Its bearing on certain general principles of heart failure Circulation I 343 1950

130 *Ikkos D Luft R* Kroppens extracellulära vätskerum. Nord Med 57 137 1957

131 *Ivanitskaya M A* Roentgen picture of nephrogenic edema of lungs. Klin. Med (Mosk.) 21 54 1943.

132 *Jackson F* The radiology of acute pulmonary oedema. Brit. Heart J 13 503, 1951

133 *Javid M* Urea—new use of an old agent. Surg Clin. N Amer 907 1958.

134 *Javid M Anderson J* The effect of urea on cerebrospinal fluid pressure in monkey before and after bilateral nephrectomy J Lab. clin Med. 53 484 1959

135 *Javid M Settlage P* Effect of urea on cerebrospinal fluid pressure in human subjects. J Amer med. Ass. 160 943, 1956.

136. *Jepsen O L Lindhardtsen F* Röntgen forandringar ved akut lungedem. Fremkaldt ved transfusionsbehandling Ugeskr Laeg. 124 827 1962.

137 *J hnsson S* Expérimental p oduction and pre ention of acute edema of the lungs in rabbits. Proc. Soc. exp. Biol. (N Y) 25 181 1927

138. *Josue O Bloch L* Oedème aigu du poumon expérimental par injection intra ei neuse d'extraits de couche corticale de capsules surrénales. B ll. Soc Méd. Paris. 26 55 1908.

139 *Jung S* Zucht und Haltung der wichtigsten Laboratori m ersuchstl re VEB Gu t Fischer Ve lag Jena. 1958.

140 *Kabanchik V Kleimans M* Pulmon uremico Pren méd. argent. 38 2143, 1951

141 *Karanen A Lah sman R* The ffect of blood transfu ion and fluid therapy on the vital capacity and blood circulati n tim in uremic patients. Acta med. scand. 172 565, 1962

142. *Kassirer J P Schwart B B* Acute gl merulon phritis. New Engl J Med 265 686, 1961

143 *Keyriläinen T O Paasonen, M K.* Pulmonary edema produced by cervical vagotomy Ann. Med. exp Fenn. 36 87 1958.

144 *Kh minkov I C Kalnoon S S* X ray diagnosis of nephrogenic pulmonary edema. Urologiia 26 17 1961

145. *Klima R Rosegger H* Eigenartige Krankenheitsbilder auf Ige von Lungenödem bei Niereninsuffiziens. Med. Klin. 32 65, 1936.

146. *Knoll P.* Bemerkungen zur Infusion blut warmer physiologischer Kochsalzlösung in das Gefässystem. Arch. exp. Path. Pharmak. 36 293 1895

147 *Koenig H Koenig R* Studies on the pathogenesis of ammonium pulmonary ed ma. Amer J Physiol. 158 1 1949

148. *K hler D., Barbe M* Variation des liquides d poumon dans l oedème pul monaire aigu d origine barbiturique Com paraison avec l oedème adrénalique C. R. Biol. (Paris) 148 290 1954

149 *Korner P L* The effect of noradrenaline —induced systemic vasoconstriction on the formation of pulmonary edema. Aust. J exp Biol. med. Sci 31 405, 1953.

150 *Kraus F* Über Lungenödem. Z. exp. Path. The 14 402, 1913.

151 *K umbhaa E. B Chanutin L* Studies on e perimental plethora in dogs and rabbits. J exp. Med. 35 817 1922.

152 *Lagrange E. Pomel R* Recherches perimentales sur l'oedème pulmon ire Arch. int Pharmacodyn, 79 445 1949

153. *Lands A M Gatting R A Larson P S* The size of the tracellular fluid compartment bef re and after massive infusions. Am r J Physl l. 130 421 1940.

154 *Landst iner K* Über Agglutinationser scheinungen normalen m nschli hen Blutes Wien. klin. Wschr 14 1132 1901

155 *Langendorf R Pick A* El ktrokardiogramm bei ak ter nephriti Acta med scand. 91 1 1938

156. *La t J M M Donald C O J nes R A B nd E E* Rate of eq ilibration of inuli nd man itol between pla ma nd i terstitial water in edem tou tates. J Lab. lin. Med. 39 62, 1952.

157 *Lavari J L, S brow L pr J L* The

pathogenesis of pulmonary edema. Sovets. Med. 24 97 1960

158 Lepelleis M Experiences sur le principe de la le p 242 D'Hautel, Paris 1812

159 Legendre: Quoted from Luisada, 1940.

160 Leleng M Bernard J L'image radiologique d'un cas d'oedème aigu du poumon d'origine rénale chez un enfant. Soc. Med. Hôp Pari 177 185, 1937

161 Lepeschkin E. Das Elektrokardiogram. Verlag Theodor St Inkopff Dresden, 1947

162 Levine H D Spontaneous changes in the normal rabbit electrocardiogram. Amer Heart J 24 209 1042.

163 Lerow P Slysted H Glomerulonefriti with initial lung-purpura. Acta med. scand. 163 403 1960.

164 Larbe V Lung edema following bilateral agotomy J. exp. Med. 70 117 1939.

165 Lower R Tractatus de Corde. London, 1680 (Quoted from Short 1944)

166 Lower R Philosoph. Tra s. Royal Soc. London, 1 128 1665—1666. (Quoted from Short 1944)

167 Luisada, A Beitrag zur Pathogenese und Therapie des Lungödems und des Asthma cardiale Arch. exp. Path Pharmak. 132 313, 1928.

168 Luisada A. The pathogenesis of paroxysmal pulmonary edema. Medicine 19 475, 1940.

169 Luisada, A.: Therapy of paroxysmal pulmonary edema by antifoaming agent Circulation 2 872, 1950

170 Luisada, A Sarnoff S J Paroxysmal pulmonary edema consequent t timulation of cardiov scular receptors I II III. Am. Heart. J 31 270 1946

171 Lunderquist A (I collaboration with N. Alwall, A Tornberg) On the artificial Kidney XXI The efficacy of the dialyser —ultrafilter intended for huma se including preliminary report on tre t ment of oedemae patient by mean of ultrafiltration. Acta med. scand 143 307 1952.

171a Lunderquist A Kon tgjord pure. VIII Ultraf ltrationsbehandling id perimentell Iiskelunga hos kanin. Nord Med 50 1319 1953.

172 Lhoit M Über die Entst hung des Lungödems. Beitr path. Anat 14 401 1893.

173 MacKeen, A D Symposium on oedema. III Review of the patho-physiology of pulmonary oedema. N S. med. Bull. 39 332, 1960.

174 Macht D L., Ting G. C.: The ffect of some polyhydric alcohol on the beha iou of rats in the circula maze Amer Phy iol. 60 496, 1922.

175 Maleit Quoted from Luisada 1940

176 Manery J F Hastings, A. B The distribution of electr lytes in mammalia tis sues J Biol. Chem. 127 657 1939

177 Marz, G. F Keraring J W O Lin, L R Dilational hypervolemia during tra ru thral resection of the prostate. J Amer med. Ass. 174 1834 1960

178 Marz, G F Ratheas, R O Li L. R. I tra enous infusions of 10 per cent cytol, 5 per cent dextrose/water or dextran in dogs. J Urol (Baltimore) 81 421 1960

179 Mayerson, S Patterson, R. M M Kee A Le Brie S J Mayerson, P Permeabil ty of lymphatic vessels. Amer J Physiol. 201 98, 1962

180 Mendel R M Ramarino P M Gerell B W ter sodium nd potassium content of human, guinea pig and rabbit lung Proc Soc. xp Biol. 82 318, 1953

181 Merklen, P Oedema pulmonaire et uremie C. R XIII Congr I ternat. Med. Paris 4 219 1900

182 Merrill J P Smith, S., Callahan E. F Thorn, G W The use of an rtificial kidney II Clinical periences J clin. I estig 29 425, 1950

183 Mill J Matthew S. A A tudy of the mechanical factors in experimental acute pulmonary edema Intern. Int. Med. 4 236, 1909

184 Mollison, P L Blood tra sfusion cli ical medicine Blackwell Scientific Publications, Oxford 1961

185 Moon, V H Morgan, D R Experimental pulmonary edema. Arch. P th. 21 ,85 1936

186 Mora Y Hemodynamic and electrolyte tudies in acut glomerulonephritis Clin Res. Proc 5 206, 1957

187 *Morrison, A E., Lundy J S Esser H E* An evolution of replacement fluids in laboratory animals following control hemorrhage. Circulation 5 208, 1952.

188. *Mulrow P J., Oestreich H M Swan, R C* Measurement of extracellular fluid volume of nephrectomized dogs with mannitol, sucrose, thiosulfate and radiosulfate Amer J Physiol *185* 179 1956.

189 *Murphy F D Correl H Grill J C.:* The effects of intravenous solutions on patients with and without cardiovascular defects. J Amer med. Ass. *116* 104 1941

190 *Müller W.,* Die Abhängigkeit des arteriellen Druckes von der Blutmenge. Arbeiten aus der physiol. Anstalt zu Leipzig, p. 159 1874

191 *Müller H* Physiologie und Klinik der Bluttransfusion. Gustav Fischer Verlag, *Jena 1960*

192. *Nairn R C.,* Oedema and capillary anoxia. J Path. *63* 213, 1951

193 *Nemir R L. Bernbaum, S L.,* Pulmonary edema occurring during the course of renal azotemia. J Dis. Child. *95* 516 1958.

194 *Nessa C B Rigler L G* The roentgenological manifestations of pulmonary edema Radiology *37* 35 1941

195 *Nickel J F Lawrence V Leifer D B Bradley S E* Renal function electrolyt excretion and body fluids in patients with chronic renal insufficiency before and after sodium deprivation. J clin. Invest. *32* 68, 1953.

196. *Nieth H* Bedrohliche Situation bei akuter Glomerulonephritis. Internist *2* 45 1961

197 *Olsson O* Some radiological problems connected with Bright's disease Brit. J Radiol. *27* 88, *1954*

198. *Olsson O* Roentgen examination of th kidney and the ureter Encyclopedia of urology vol 5, edited by Aiken et al. Springer Verlag, Berlin 1962.

199 *Opdyke D F Duomarco J Dillon, W H Schreiber H Little R C Seely R D* Study of simultaneous right and left atrial pressure pulses under normal and experimentally altered conditi ns. Amer J Physiol. *154* 258, 1948.

200. *Paasonen M K* Effect of some antihistaminics on adrenaline responses in animal tests. Ann. Med. exp. Fenn. 31 suppl. 7 1953

201 *Pace N Kline L., Schachman, H K Harfenist M* Studies on body composition. IV Use of radioactive hydrogen for measurement in vivo of total body water J Biol. Chem. *168* 459 1947

202. *Paine R Butcher H R Howard F A., Smith A B Smith, J R* Observations on mechanisms of edema formation in the lungs. J Lab. clin. Med. *34* 1544 *1949*

203. *Painter E. E* Total body water in the dog Amer J Physiol. *129* 744 1940.

204 *Pattle R. E.* Failure of local anoxia in rabbit lung to produce or intensify pulmonary oedema. J Path. Bact. *72* 211 1956.

205. *Perret L., Kahlbäck B* Lungförändringar vid akut nefrit. Finska Läk.-Sällsk. Handl. *99* 187 1956.

206. *Poulsen, T.,* Quantitative estimation of pulmonary oedema in mice Acta pharmacol. (kbh) *10* 117 1954.

207 *Poulsen, T* Investigations into the pulmonary oedema produced in mice by carbon dioxide Acta pharmacol. (kbh) *10* 201 1954

208. *Poulsen T.,* Experimental investigations into the effect of morphine and allypropymal on *carbon-dioxide provoked* pulmonary oedema in mice. Acta pharmacol. (kbh) *10* 246, 1954

209 *Poulsen, T* Experimental investigations into the effect of ether on carbon-dioxide provoked pulmonary oedema in mice. Acta pharmacol. (kbh) *10* 253, 1954

210 *Poulsen T* Experimental investigations into the effect of atropine, sympatholytics, sodium nitrate and promethazine on pulmonary oedema provoked by carbon-dioxide. *Acta pharmacol.* (kbh) *10* 379 1954

211 *Poulsen T* Kuldioxidets anaestecerande och lungedemframkaldende virkning. Copenhagen 1956.

212. *Prichard M M L., Daniel P M Ardran G M* Peripheral ischaemia of the lung Brit. J Radiol. 27: 93 1954

213. *Randall R. E., Glazier J S Liggett M.,*

Nephritis with lung haemorrhage. Lancet 1 690, 1963.

213 a. *Rappaport F* Rapid microchemical methods for blood and CSF examinations. Grune sch Station, Inc. New York 1949.

214. *Reichsman, F* Studies on the pathogenesis of pulmonary edema following bilateral vagotomy Am. Heart J 31 590, 1946.

215. *Rendich, R. A., Leng A. H Cove A. M.:* Pulmonary manifestations of azotemia. Am. J Roentgenol. 46 802, 1941

216 *Renner, S.:* On the roentgenological picture of pulmonary edema. Acta radiol. (Stockh.) 30 169 1948.

217 *Rieker G.* Die Therapie des Lungenödems. Internist (Berl.) 1 79 1960.

218 *Riechert W* Beitrag zum Cardiac I Lungenödem. Arch. exp. Path. Pharmak. 197 639 1941.

219 *Roberts J O* Urea as an agent for the reduction of intra-cranial pressure. (Quoted from Javid & Settlage 1956.)

220 *Rolls, J O* Urea is not equally distributed between the water of the blood cells and that of the plasma. J Biol. Chem. 151 329 1943.

221 *Rothlin, E.:* Experimenteller Beitrag zur Pathologie und Therapie der Spätfolgen der durch Phosgen erzeugten Lungenödems. Schweiz. med. Wschr 70 641, 1940.

222. *Roubier C., Plauchu M* Sur certains aspects radiographiques de l'oedème pulmonaire chez les cardio-rénaux azotemiques. Arch. med. chir Appar resp. 3 189 1934.

223. *Schiano S D'Onofrio T* Ricerche sull' uremia perikorniale. Sperimentale 107 423, 1957

224. *Shafer E. S.* Experiments on the cervical vagus and sympathetic. Quart J exp. Physiol. 12 231, 1920

225. *Shiff M* Gesammelte Beiträge zur Physiologie. I. p. 330—410. B. Benda Lausanne, 1894.

226 *Schwartz, I L., Bredd E. S Maxwell M H* Comparison of the volume of distribution, renal and extrarenal clearances of inulin and mannitol in man. J clin. Invest. 29 517 1950.

227 *Schwanenberg, I A.:* The study of protein metabolism of the transudate in some types of experimental pulmonary edema. Pat. Fiziol. exp. Ter 4 60, 1960.

228. *Shafer E. S* Experiments on the cervical vagus and sympathetic. Quart. J exp. Physiol. 12 231, 1920.

229 *Sharpey-Shafer E. P* Transfusion and the anaemic heart. Lancet 2 296, 1945.

230. *Sharpey-Shafer E. P Wallace J.:* Circulatory overloading following rapid intravenous injections. Brit. Med. J 2 304, 1942.

231 *Short R. H D* Pulmonary changes in rabbits produced by bilateral vagotomy J Path. 56 355, 1944

232. *Sjöstrand T* On the principles for the distribution of the blood in the peripheral vascular system. Skand. Arch. Physiol. 71 suppl 5 1935.

233. *Smith H W* Principles of renal physiology Oxford University Press. Inc. New York 1956.

234. *Smith, W W Finkelstein, N Smith, H W* Renal excretion of hexitols (sorbitol, mannitol and dulcitol) and their derivatives (sorbitan, isomannide and sorbide) and of endogenous creatinine-like chromogen in dog and man. J Biol. Chem. 135 231 1940.

235 *Steffensen, K. A.* Some determination of the total body water in man by means of intravenous injections of urea. Acta physiol. scand. 13 282, 1947

236 *Stricke M H* Experimental uremic-uremic enteritis. Arch. intern. Med. 42 835, 1928.

237 *Stone C. A., Loew E. R.:* Effect of various drugs on epinephrine induced pulmonary edema in rabbits. Proc. Soc. exp. Biol. (N Y) 71 122, 1949.

238 *Tessier J* De l'oedème aigu du poumon. C. R. XIII Congr Internat. Med. Paris 4 190 Masson, Paris 1900.

239. *Thölen, H* Urämische Flüssigkeitslunge und hypertensive Encephalopathie. Bull. schweiz. Akad. med. Wiss. 15 396, 1959—60.

187 *Morrison, A. E., Landy J S Essex H E* An evolution of replacement fluids in laboratory animals following control hemorrhage Circulation 5 205, 1952.

188. *Mulrow P J Oestreich H M Swan, R C* Measurement of extracellular fluid volume of nephrectomized dogs with mannitol, sucrose thiosulfate and radiosulfate Amer J Physiol. *185* 179 1956.

189 *Murphy F D Correl H., Grill J C* The effects of intravenous solutions on patients with and without cardiovascular defects. J Amer med. Ass. *116:* 104 1941

190 *Müller W* Die Abhängigkeit des arteriellen Druckes von der Blutmenge. Arbeiten aus der physiol Anstalt zu Leipzig p. *169 1874*

191 *Möller H.* Physiologie und Klinik der Bluttransfusion Gustav Fischer Verlag, Jena 1960

192. *Nairn R C.* Oedema and capillary anoxia. J Path. *63* 213, 1951

193. *Nemir R L. Bernbaum S L.* Pulmonary edema occurring during the course of renal azotemia J Dis. Child. *95* 516, 1958.

194 *Nessa C B Rigler L G* The roentgenological manifestations of pulmonary edema. Radiology *37* 35, *1941*

195 *Nickel J F Lawrence W Leifer D B Bradley S E* Renal function electrolyte excretion and body fluids in patients with chronic renal insufficiency before and after sodium deprivation. J clin. Invest. *32* 68, 1953

196 *Nieth H* Bedrohliche Situation bei akuter Glomerulonephritis. Internist 2 45, 1961

197 *Olsson O* Some radiological problems connected with Bright s disease. Brit J Radiol. 27 86 1954

198. *Olsson O* Roentgen examination of the kidney and the uret r Encyclopedia of *urology* ol. *5*, edited by Alken et al. Springer Verlag Berlin 1962.

199 *Opdyke D F Du marco J Dillon, W H Schreiber H Little R C Seely R D* Study of simultaneous right and l ft atrial pressure pulses under normal and experimentally altered conditions. *Amer* J Physiol. *154* 258, 1948

200. *Paasonen, M K* Effect of som anti histaminics on adrenaline responses in animal tests. Ann. Med. exp. Fenn. *31* *suppl. 7 1953*

201. *Pace N Kline L., Schachman, H K Harfenist M* Studies on body composition. IV Use of radioactive hydrogen for mea surement in vivo of total body water J Biol. Chem. *168* 459 1947

202. *Paine R Butcher H R Howard F A., Smith A. B Smith, J R* Observations on mechanisms of edema formation in the lungs. J Lab. clin. Med. *34* 1544 1949

203. *Paint r E. E.* Total body water in the *dog Amer J Physiol. 129 744 1940.*

204 *Pattle R. E.* Failure of local anoxia in rabbit lung to produce or intensify pul monary oedema. J Path. Bact. *72* 211 1956.

205. *Perret L., Kuhlbäck B* Lungförändring ar vid akut nefrit. Finska Läk. Sällsk. Handl *99* 187 1956.

206. *Poulsen T* Quantitative estimation of pulmonary oedema in mice Acta pharmacol (kbh) *10* 117 1954

207 *Poulsen, T* Investigations into the pulmonary oedema produced in mice by carbon dioxide Acta pharmacol. (kbh) *10* 201 1954

208. *Poulsen T* Experimental in estigations int the effect of morphine and ally propymal on carbon-dio ide provoked pulmonary oedema in mice. Acta pharmacol. (kbh) *10* 216, 1954

209 *P ulsen T* Experimental investigations into the effect of ether on carbon-dioxide provoked pulmonary oedema in mice. Acta pharmacol. (kbh) *10* 253, 1954.

210 *Poulsen, T* Experimental investigations into the effect of atropine sympatholy tics, sodium nitrate and promethazine on pulmonary oedema provoked by carbon-dioxide Acta pharmacol. (kbh) *10* 379 1954

211 *Poulsen T* Kuldioxidets anaestecerande och lungedemframkaldende virkning. Copenhagen 1956.

212 *Prichard M M L., Daniel P M Ardran, G M* Peripheral ischaemia of the lung Brit. J Radiol. 27 93 1954

213. *Randall R E Gla ier J S Liggett M*

Nephritis with lung haemorrhage Lancet 1 499, 1962.

213 a. *Rappaport F* Rapid microchemical methods for blood and CSF examinations. Grune ech Station, Inc. New York 1949.

214. *Reichsman, F* Studies on the pathogenesis of pulmonary edema following bilateral vagotomy Am. Heart J 31 590, 1946.

215. *Rendich, R. A., Leng, A. H Cove A. M* Pulmonary manifestations of azotemia. Am. J Roentgenol. 46 802, 1941.

216. *Renner S.*: On the roentgenological picture of pulmonary edema. Acta radiol. (Stockh.) 30 169 1948.

217 *Reiter G* Die Therapie des Lungenödems. Internist (Berl.) 1 29, 1960.

218 *Rieckert W* Beitrag zum Cardiacol-Lungenödem. Arch. exp. Path. Pharmak. 197 620, 1941.

219. *Robert J O* Urea as an agent for the reduction of intra-cranial pressure. (Quoted from Javid & Settlage 1956.)

220 *Rolls J O* Urea is not equally distributed between the water of the blood cells and that of the plasma J Biol. Chem. 151 529 1943.

221. *Rothlin, E.* Experimenteller Beitrag zu Pathologie und Therapie der Spätfolgen der durch Phosgen erzeugten Lungenödems Schweiz. med. Wschr 70 641 1940

222. *Roubier C., Plauchen, M* Sur certain aspects radiographiques de l'oedème pulmonaire chez les cardio-rénaux azotémiques. Arch. med chir Appar resp. 3 180 1934.

223 *Schiano S., D'Onofri T* Ricerche sull' uremia sperimentale. Sperimentale 107 435, 1957

224 *Shafer E. S.*: Experiments on the cervical agus and ympathetic. Quart. J exp. Physiol 12 231 1920

225 *Sheff M* Gesammelte Beiträge zur Physiologie. I. p. 320—410. B. Benda Lausanne 1894.

226 *Schwartz, I L., Breed E. S Maxwell, M H* Comparison of the volume of distribution, renal and extrarenal clearances of inulin and mannitol in man. J clin. Invest. 29 517 1950

227 *Serebrovskaya, I A.* The tudy of protein metabolism of the tran udate in some types of experimental pulmonary edema. Pat. Fiziol. exp. Ter 4 60 1960.

228. *Shaf E. S* Experiment on the cervical gus and ympathetic. Quart. J exp. Physiol. 12 231 1920.

229 *Sharpey-Shafer E. P.*: Transfusion and the anaemic heart. Lancet 2 296, 1945.

230 *Sharpey-Shafer E. P Wallac J* Circulatory overloading following rapid intravenous injections. Brit. Med. J 2 301 1942.

231 *Short, R H D* Pulmonary changes in rabbits produced by bilateral vagotomy J Path. 56 355, 1944.

232. *Sjöstrand T* On the principles for the distribution of the blood in the peripheral vascula system. Skand. Arch. Physiol. 71 suppl. 5, 1935.

233. *Smith, H W.*: Principles of renal physiology Oxford University Press. Inc. New York 1956.

234. *Smith, W W Finkelstein, N Smith, H W* Renal excretion of hexitol (sorbitol, mannitol and dulcitol) and their derivatives (sorbitan, isomannide and sorbide) and of endogenous creatinine-like chromogen in dog and man. J Biol. Chem. 135 231 1940.

235. *Steffensen, K A.*: Some determination of the total body water in man by means of intravenous injections of urea. Acta phy siol. scand. 13 282, 1947

236 *Steicher M H.*: Experimental uremia-uremic enteritis. Arch. intern. Med. 42, 835, 1928.

237 *Stone C. A., Loew E. R.*: Effect of various drugs on epinephrine induced pulmonary edema in rabbits. Proc. Soc. exp. Biol. (N Y.) 71 122, 1949.

238. *Teissier J* De l'oedème aigu du poumon. C. R. XIII Congr Internat. Med. Paris 4 100, M sson, Paris 1900.

239. *Thölen, H* U Inbeche Flüssigkeitsbilungs und hypertensive Encephalopathie. Bull. schweiz. Akad med. Wiss. 16 306 1960 —60

240 *Traube L.* Gesammelte Beiträge zur Pathologie und Physiologie. I p 1—135, August Hirschwald. Berlin 1871

240 a. *Tybjærg Hansen A* Osmotic pressure effect of the red blood cells—possible physiological significance Nature *190* 504 1961

241 *Udvardy L.* Form und Grössenveränderungen des Herzens bei Nierenenkrankungen Fortschr Röntegnstr *54* 559 1930.

242 *Valsalva A M V* Opera, Venice, Epistola XIII art. 30 1740 (Quoted from *Legallois 1812.*)

243 *Vanpeperstraete F.* Effect de la trachéotomie et de l aspiration bronchique sur l'oedème pulmonaire algu adrénalinique du lapin. C. R Soc. Biol. (Paris) *151* 2 1957

244 *Vanquelin & Segales* Quoted from Streicher 1928.

245 *Vieussens R.* Trait du Coeur p 122, Toulouse 1715 (Quoted from Legallois.)

246. *Visscher M B Haddy F J Stephens G* The physiology and pharmacology of lung edema. Pharmacol. Re *8* 389 1956.

247 *Warthen, H J* Massive intravenous injections. Arch. Surg *30* 199 1935

248 *Wasserman K Loeb L., Mayerson, H S* Capillary permeability to macromolecules. Circulat Res. *3* 594 1955.

249 *Weed L. H McKibben, P S* Pressure changes in the cerebro-spinal fluid f llowing intravenous injecti n of solutions of vari u concentrations. Amer J Physiol. *48* 512, 1919

250 *Welch W H* Zur Pathologie des Lungenödems. Virchows Arch. path. Amer *72* 375, 1878.

251 *Werkenthin M* The roentgenological aspect of lung edema Am. J Roentgen l. *41 183 1939*

252. *Westermark N* Roentgen studies of the lungs and heart. The University of Minnesota Press Minneapolis, 1948.

253. *Whitehall M R Langcope W T Williams R* The occurrence and significance of myocardial failure in acute hemorrhagic nephritis. Bull. Johns Hopk. Hosp *64* 83 1939

254 *Wiggers C J.* Physiology in health and disease p. 695, Lea & Febiger Philadelphia 1939

255. *Wils n J G* Pulmonary oedema in acute glomerulonephritis. Arch. Dis. Child. *36* 661 1961

256. *Wood E H Moe G K* The measurement of edema in the heart lung preparation Amer J Physiol. *136* 500 1942.

257 *Yeomans A. Porte R R., Swank R. L.* Observati ns on certain manifestations of circulatory congestion produced in dogs by rapid infusion. J clin. Invest. *22* 33, 1943.

258. *Y ung I E Pearce J E Steven n J A.* Renal responses to hypervolemia in the dog Canad. J Biochem. *33* 800 1955

259 *Zaldivar R. V C Farinas L.* Pulmon azotemico o edemea *subagudo d l pul* mon en los renales. Rev Cuba Cardiol *14* 141 1954.

260 *Zdansky E.* Beiträge zur Kenntni der kardialen Lungen I nung auf Grund röntgenologischer Kli ischer und anatomischer Untersuch ngen. Wiener Arch inn. Med. *18* 461 1929

261 *Zdansky E.* Über das Röntgenbild des Lungenödems, gleichzeitig ein Beitrag zur Frage der Pathogenese des Lungenödems. Rö tgenpraxis, *5* 248, 1933.

262. *Zettergren, L.* Uremic Lung Acta Soc Med. upsalien *60* 161 1955.

240 *Traube L.* Gesammelte Beiträge zur Pa thologie und Physiologie L p 1—135, August Hirschwald. Berlin 1871

240 a. *Tybjærg Hansen, A* Osmotic pressure effect of the red blood cells—possible physiological significance *Nature 190* 504 1961

241 *Udvardy L.* Form und Grössenveränder ungen des Herzens bei Nierenenkrankun gen. Fortschr Röntegnatr *54* 559 1936.

242 *Valsalva A M V* Opera, Venice, Epi stola XIII art. *30* 1740 (Quoted from Legallois 1812)

243. *Vanpeperstraete F* Effect de la trachéotomie et de l'aspiration bronchique sur l oedème pulmonaire aigu adrénalinique du lapin. C. R Soc. Biol. (Paris) *151* 2, 1957

244 *Vanquelin & Segales* Quoted from Strel cher 1928.

245 *Vieu ens R* Traite du Coeur p 122, Toulouse 1715 (Quoted from Legallois.)

246. *Visscher M B Haddy F J Stephens G* Th physiology and pharmacology of lung edema Pharmacol. Rev *8* 389 1956.

247 *Warthen, H J* Massive intravenous in jections Arch Surg *30* 199 1935

248. *Wasserman K Loeb L Mayers n, H S* Capillary permeability to macromolecules. Circulat. Res. *3* 504 1955.

249 *Weed L. H McKibben, P S* Pressure changes in the cerebro-spinal fluid fol lowing intravenous injection of sol tions of various concentrations. Amer J Phy siol. *48* 512, 1919

250 *Welch W H* Zur Pathologie des Lun genödems. Virchows Arch path Amer *72* 375, 1878.

251 *Werkenthin M* The roentgenological aspect of lung edema. Am J Roentgenol *41* 183, 1939

252 *Weste mark N* Roentgen studies of the lungs and heart. The University of Min nesota Press. Minneapolis 1948.

253. *Whitehall M R Langcope W T Wil liams R* The occurrence and significance of myocardial failure in acute hemorr hagic nephritis. Bull. Johns Hopk. Hosp *64* 83, 1939

254. *Wiggers C J* Physiology in health and disease p 605, Lea & Febiger Philadel phia 1939

255 *Wils n, J G* Pulmonary oedema in acute glomerul nephritis. Arch. Dis. Child. *36* 661 1961

256. *Wood E H Moe G K* The measure ment f edema in the heart-lung prepara tion. Amer J Physiol. *136* 506 1942.

257 *Yeomans A., Porte R. R Swanl R. L.* Observations on certain manifestations of circulatory congestion produced in dogs by rapid infusion. J clin. In est. *22* 33, 1943

258. *Young I E Pearce J E Stevenson, J A.* Renal responses to hypervolemia in the dog Canad. J Biochem. *33* 800 1955

259 *Zaldivar R V G Farinas L* Pulmon ar temico o edemea subagudo del pul mon en los renales. Rev Cuba Cardiol. *14* 141 1954.

260 *Zdansky E.* Beiträge zur Kenntnis der kardialen Lungenstauung uf Grund rönt gen logischer klinische und anat mi sch r Untersuchungen. Wiener Arch. inn Med. *18* 461 1929

261 *Zdansky E* Über das Rö tgenbild des *Lungenödems, gl ichz itig ein Beitrag z* Frage der Pathogenese des Lungenödems *Rö tgenpraxis, 5* 248, 1933

262. *Zetterg en, L.* Uremic Lung Acta Soc. Med. upsalien *60* 161 1955